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PLANT PHYSIOLOGY AT THE ITHACA CONGRESS

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The International Congress of Plant Sciences held at Ithaca, N. Y., August 16 to 23, 1926, was the botanical event of a lifetime to those who were fortunate enough to be present; and it is safe to assert that no group came away with more stimulating and provocative recollections of the week than the plant physiologists. Several of the papers there presented were wholesomely upsetting to accepted notions, others were helpful in the clearing up of concepts hitherto somewhat foggy, or summed up knowledge hitherto incomplete or scattered. The program committee is to be congratulated for its complete avoidance of those ten-minute soporifics that sometimes creep in despite the best-laid plans.

It was regretted by everyone that the chairman of the physiological section, Prof. LEPESCHKIN, of Charles University, Prague, could not be present to preside at the meetings. In his absence Dr. C. O. APPLEMAN, University of Maryland, presided over the discussions.

The color of the entire meeting was indicated by the first paper of the opening session, on the osmotic properties of the plant cell, by Prof. A. URSPRUNG, of the University of Fribourg, Switzerland. Prof. URSPRUNG was unable to attend in person, and arrangements had fortunately been made to have this communication read by an American associate, Prof. WILLIAM A. BECK, of the University of Dayton. Prof. URSPRUNG'S paper belonged to the "upsetting" category, and was received alertly, but in an atmosphere of general caution. It was recognized that if his results are fully borne out by further work, large sections of current standard physiological interpretation in the field of water absorption and translocation will have to be radically revised.

Prof. URSPRUNG has greatly refined the technique for studying osmotic phenomena, both in improvements in manipulation, and in the substitution

of sugar solutions for the potassium nitrate or similar salt solutions now in vogue for plasmolytic studies. He further insists that the critical point in plasmolysis is not the moment of incipient withdrawal of protoplasm from the wall of the cell, but at the beginning of diminution of distention, the first signs of shrinkage, of the fully turgid cell. Determination of plasmolytic concentrations by this method leads to radically higher values for the suction tensions in plant cells than have so far been commonly reported.

He introduces several new terms into the discussion, differentiating between the suction force of the contents of the cell, wall pressure, turgor pressure, and the suction force of the cell. The relations of three of these terms are indicated by the equation:

(Suction force of the cell) = (Suction force of the contents) − (Wall pressure).

The quantities are notionally and numerically different, and must not be designated by a common term, *e.g.*, "osmotic pressure"; furthermore, each quantity varies with the degree of saturation of the cell.

In studies on the translocation of water, a very steep gradient from root hairs to uppermost transpiring organs (leaf palisade, petals) was discovered. This gradient held not only for entire plants but also for their parts. Thus, in a row of palisade cells in an ivy leaf the minimum suction force (12.1 atmospheres) lay near the vein, and an increase was noted with the distance from the vein, up to 32.6 atmospheres.

Perhaps the most outstanding of Prof. URSPRUNG'S discoveries relates to the existence of a polarity of suction forces exerted by opposite sides of the same cell, and the effect of this polarity in certain cells of the root in carrying forward the unilateral movement of water in the plant as a whole. It was discovered that in a cross section through the zone of absorption of the root tip of *Vicia Faba,* the suction force rose from 1.1 atmospheres in the epidermis to 4.1 atmospheres in the innermost cells of the cortex, but then suddenly dropped in the endodermis to 1.9 atmospheres. In spite of its low mean suction force, the endodermis can still absorb water by virtue of the polar differentiation of the suction force. On its outer side 4.7 atmospheres were found, and on its inner side 0.5 atmosphere. Thus the endodermis acts simultaneously as a suction pump and a reduction valve. Polar differentiation is even more marked in the parenchyma cells that border on the root vessels; like a combined suction pump and force pump they can take up water on one side and press it into the vessels on the other.

The influence of climatic and edaphic factors on the suction force was also studied. It was found that the moisture of the soil plays the chief role; a heavy rain caused the suction force of the leaf of *Satureia alpina* to fall from 34.5 to 13.5 atmospheres. The relative humidity of the air

was found to be next in importance; its fluctuations on a summer day caused the corolla of *Bellis* to vary by about 10 atmospheres.

If the proper precautions be taken, the influence of habitat on the suction force can also be determined. In the Alps, for instance, the vexillum of *Lotus corniculatus* showed 9.5 atmospheres in a moist locality, 14.5 atmospheres on the alpine meadow, 29.5 atmospheres on a belt of humus, and 34.5 atmospheres on dry rubble, simultaneously. The suction force increased in this case with the reduction of the water supply. A comparison of the suction force in the plain with that in the mountains did not manifest a clear difference, and SCHIMPER's idea of the physiological dryness of a peaty substratum could not be verified.

Dr. H. S. REED, of the Citrus Experiment Station at Riverside, California, attacked the vexing problem of the significance of quantitative expressions of plant growth and differentiation. Growth and differentiation, he said, may be considered as irreversible changes in the mass of an organism through which there is an increase in size and complexity. The transformation of extraneous materials into the mass of the organism appears to proceed at a rate proportional to time.

Our knowledge of the growth of organisms has been immensely advanced in recent years by the application of quantitative methods to its study. Having found that the increase in size of an organism is an orderly change, it is possible to give mathematical expression to it, analogous to those used to express chemical processes. Biologists have not yet settled on any one equation to express the process, perhaps none yet advanced is adequate for the purpose. Their use has, however, focussed attention upon the orderly nature of the growth process and makes it more apparent that this process is a manifestation of the energy relationships prevailing in other forms of matter. The size of a plant at a given time appears to depend on the nutrient level and on the specific constant of growth. The latter is independent of environmental conditions and expresses the velocity of transformation of unit mass of nutritive material into tissue substance. The growth curves of the same race growing at different rates easily allow one to determine the validity of these constants.

A brief experience with growth curves of organisms shows the prevalence of growth cycles, which are quite independent of definite fluctuations in environmental conditions. These cycles overlap to a certain extent and it is frequently impossible to discern their exact limits. The mathematical expression of growth cycles presents no especial difficulties whether one regards them as successive or as a single process, in which the main reaction is periodically accelerated or retarded by one of the products of the reaction.

A more precise study of the processes of differentiation and development has shown the existence of causal factors which produce a relative constancy of form of individuals of a given species or variety. The size of the organs bears evidence that the process of differentiation results in a stoichiometric distribution of mass with respect to space. The precise form is dependent upon the distribution of plastic materials, the dominance of the apical meristem, the release of certain buds from dormancy, all of which appear to be correlated. The biomathematical aspects of the physiological problems have become increasingly important.

Dr. V. LUBIMENKO, of the Leningrad Botanic Garden, offered a masterly summing-up of our present knowledge of the composition and genesis of chlorophyll, to which his own researches have been a major contribution. He holds that the food-building operations of plants began their evolution as chemosyntheses, similar to but simpler than those now familiar in certain bacteria. Photosynthesis replaced chemosynthesis after the formation of pigments in the protoplasm of saprophytic plants, the process becoming more complex as one ascends the evolutionary scale.

The formation and accumulation of chlorophyll in the algae, mosses, ferns, and gymnosperms is accomplished in the absence of light, which for these plants serves only as a stimulator. Among the angiosperms light enters directly into the reactions of the formation of chlorophyll; however, enzymatic reactions also play a part in the accumulation of chlorophyll in connection with the photochemical reactions. Leucophyll is probably a single definite albuminoid substance, and the formation of chlorophyll is intimately associated with the transformations of the albuminoid substances in the cell. The spectrocolorimetric method is most rapid and most exact for the estimation of chlorophyll in living leaves. By means of this method it has been established that the maximum amount of chlorophyll elaborated by various species is a constant and represents an hereditary character. The application of illumination of feeble intensity is manifested by an increase of the pigments in the plastids; this phenomenon has been observed not only with terrestrial plants but also with marine algae. Among the red algae the quantity of phycoerythrin increases in greater proportion than the quantity of chlorophyll with the depth of habitation. The number of species rich in chlorophyll among land plants increases in the north and attains a maximum at the equator.

The hypotheses advanced by various writers on the chemistry of photosynthesis are inadequate, for they do not take into account completely the condition of the chlorophyll in the plastids. In every case it is certain that the synthesis of the organic materials in the plastid includes not only photochemical reactions, but also enzymatic reactions. This is the reason there is no simple proportionality between the energy of photosynthesis and the

quantity of chlorophyll in the leaves. A maximum energy of photosynthesis corresponds to an optimum quantity of chlorophyll. Under natural conditions it is low light intensity or temperature which is able to limit the energy of photosynthesis. In general plants do not suffer from insufficient CO_2 in the atmosphere, for the energy of the photochlorophyllous work of the leaves is conditioned and limited by internal factors, and notably by the speed of transport of the substances assimilated by the leaves during the day. Upon these internal factors depends the adaptation of the plants to intensity and periodicity of illumination.

It is very probable that it is the relative energy of oxidations and reductions which determines in the end the complete course of the organic syntheses in the plant cells. The energy of these enzymatic processes determines the physiological condition of the plastids, as well as the qualitative and quantitative evolution of the pigments. Therefore a searching study of the enzymic activity of the plastids promises to clarify the fundamental problem of plant physiology—the problem of photosynthesis.

Dr. LUBIMENKO showed specimens of aqueous chlorophyll extracts, which had proved to be stable through long periods of time. He also demonstrated his spectrocolorimetric apparatus for the quantitative study of chlorophyll in the living plant.

Prof. B. E. LIVINGSTON, of Johns Hopkins University, addressed the section on the importance of viewing the environment, no less than the plant, as a dynamic complex. Physiologists have long been accustomed to look upon the plant as a mechanism receiving material and energy from its environment and delivering the material and dynamic products of its activities back to the environment. This view of the organism as solely active and the environment as solely passive is a one-sided picture. Matter and energy pass into the receptive plant by virtue of a potential with which they are already endowed, and the removal of by-products from the plant is also a function that is at least partly active. These positive physiological functions of the environment are susceptible of quantitative study, and beginnings in this direction have been made, especially in the examination of the evaporating power of the air, and of oxygen, CO_2, and water supplying power of the soil.

On the afternoon of August 17, the physiologists, horticulturists, and geneticists met with the agronomists for a round table discussion of the place of statistics in the interpretation of experimental data. The leader of the discussion was Dr. H. H. LOVE, of Cornell University. Space will not permit presentation of the various points of view expressed on this important subject. Application of statistical methods to physiological research would often prevent the drawing of erroneous conclusions from inadequate data.

On Wednesday morning, in the absence of Prof. LEPESCHKIN, the discussion was opened by Dr. W. J. ROBBINS, of the University of Missouri, who discussed the isoelectric points for plant protoplasm and their significance. The paper was an excellent summary of the recent work done on isoelectric points in living tissues, much of which has been done in the Missouri laboratory. Following this paper, Dr. L. MICHAELIS, of the University of Berlin, presented a brilliant paper on permeability of vegetable membranes and an artificial model for these membranes before an audience that taxed the meeting place to the limit. The summary of this paper was prepared for PLANT PHYSIOLOGY by Dr. MICHAELIS, and is presented in his own words.

"It has been shown by JACQUES LOEB and R. BEUTNER that the integuments of many plants in contact with electrolyte solutions establish an electric potential difference, the amount of which depends on both the nature and the concentration of the dissolved electrolytes. This effect can be most easily studied on the intact peel of an apple, but the leaf of *Plantago* also may be recommended. The effect is due to the intact wax cuticle of the surface. The P.D. depends on the concentration of a given electrolyte, in that, by a change by one power of 10 of the concentration of any electrolyte the cation of which is univalent, it will produce a change of the potential by 57 millivolts as a maximum. Such a skin therefore behaves like a metallic electrode reversible for the cation of the electrolyte dissolved in the aqueous solution.

"BEUTNER tried to explain this effect according to HABER'S theory of potential differences at interfaces of different phases. However, the explanation is easier on the basis of a certain artificial model of membrane which gives the same effect in any regard, and can only be explained by the theory of diffusion-potentials. This model is a membrane of completely dried collodion. It can be shown that this is approximately impermeable for any anion. The cation can penetrate the membrane, but the specific velocities of the different sorts of cation are much more different from each other than in an ordinary aqueous solution. The greatest difference of velocities for aqueous solutions is the difference in mobility of H^+ and Li^+ (about 10 to 1). Within the pores of the collodion membrane this ratio of velocities sometimes amounts to 1000 to 1. There is even a considerable difference in the mobilities of K^+ and Na^+.

"Diffusion of electrolytes across the apple skin can practically take place only if cations can be exchanged across the membrane, but not when the apple is dipped into pure water. All of these properties depend on the negative electric charge of collodion, and the exceedingly small pore size. There is no other effect than one of a sieve the holes of which exceed the

molecular size by only a little. The permeability depends on the molecular size, the sign of charge, and the amount of water carried by the ion.

"The plasma membrane differs from this cuticle by the fact that the structure and pore size of collodion (apple skin, etc.) are practically independent of the surrounding solution, while the real plasma membrane can be easily changed (coagulated or peptized) by electrolytes, especially by calcium. The problem of the permeability of the plasma membrane consists therefore of two different problems:

"(1) How is the colloidal structure influenced by the surrounding. solution?

"(2) What is the permeability of a membrane of a given structure for the different ions of the surrounding solution?

"The apple skin and the collodion model give us a nearly complete understanding of the second question. The problem presented by the first question, however, is not touched by these investigations. The combination of these two problems may give complete understanding of the permeability of the plasma membrane of living cells."

The meeting of Wednesday morning closed with a discussion of the mechanism of stomatal movements. This has been one of the most fascinating subjects to plant physiologists. A great deal of work has been done on it, nevertheless it has remained somewhat of a mystery. Prof. G. W. SCARTH, of McGill University, has found a correlation between the movements of stomata as well as other plant cells and organs, and the hydrogen ion concentration. Leaf sections in solutions whose P_H was subject to control showed minimal stomatal opening at P_H 5.5, and changed little until the neutral point was reached, but then opened rapidly. There is also a correlation between amylysis and degree of closure. Starch is abundant at the P_H of closure (maximum starch at about P_H 5.0) and it dwindles in amount if the guard cells become more acid, but especially on the alkaline side of neutrality. In spite of these correlations, however, synthesis and hydrolysis of starch do not wholly account for the stomatal movements; for movements occur with changes of P_H much more quickly than any change in starch can occur. Also, even when the starch is all hydrolyzed, still changes in P_H cause changes in turgor, and movements. Beautiful photomicrographs were used to show that the effective mechanism of movement is concerned with hydrophilic colloids of amphoteric nature in the guard cells, supplemented possibly by the hydrolysis and synthesis of the carbohydrates. Open stomata are alkaline in *Pelargonium,* and closed stomata acid. The alkalinity of guard cells during photosynthesis is believed to be due to the use of the CO_2 by the chloroplasts of the guard cells, and the acidity which causes closure is the consequence of higher CO_2 pressure in

the guard cell when photosynthesis ceases and respiration continues. Openings at night may be due to development of hyperacidity, which also causes hydration of the colloidal material involved in the production of turgor. The speed with which opening and closing takes place on access or exclusion of light indicates that under normal as well as under experimental conditions, the primary mechanism through which the H-ion increase or decrease acts, is the hydration of the vacuolar colloid rather than hydrolysis of starch. Thus this problem nears a complete physico-chemical solution.

On Thursday morning the physiologists met jointly with the horticulturists, and in the afternoon with the horticulturists and ecologists for a round table discussion of the factors influencing flowering and fruiting *versus* vegetation. Naturally much attention was given to the well known work of GARNER and ALLARD on photoperiodism, and the discoveries which have grown up out of their recognition of the powerful influence of the illumination period on flowering and other events in the life cycle of plants.

Professor AUCHTER, of the University of Maryland, discussed the influence of shading on the behavior of apple trees, and Prof. H. C. THOMPSON, of Cornell, presented a most interesting study of temperature effects on flowering of celery. Early chilling induced premature flowering of this crop. The physiological significance of carbohydrate accumulation as a main cause of dormancy was presented by Dr. H. D. HOOKER, of the University of Missouri. One of the finest papers presented was not listed on the program, a paper by Professor WALLACE, of the Long Ashton Station, Bristol, England, on the effects of mineral deficiency on the growth and leaf behavior, flowering and fruiting of fruit trees and woody shrubs. The Ca/K ratio was found to be important in controlling physiological breakdown of leaf tissues.

The round table discussion in the afternoon took up photoperiodism, the carbohydrate-nitrogen ratio, phosphorus, carbon dioxide enrichment, water supply, etc., as related to vegetative and fruiting activities of plants. It was featured by the presentation of data by Prof. R. H. ROBERTS, of Wisconsin, who showed that the behavior of the plant is closely correlated with the internal nutritive conditions, especially the carbohydrate-nitrogen situation.

Just as the first day's session opened with a paper of unusual interest, so also did the program of Friday, the last day of the Congress, begin with the presentation of data very disturbing to many of our cherished physiological and ecological ideas. Prof. N. A. MAXIMOW's paper left little place for the orthodox idea that xerophytes are plants of low transpiration rates and low water requirement. Many xerophytes, for instance, *Peganum*, *Zygophyllum*, and *Artemisia*, transpire intensely and possess a high water requirement. On the contrary, shade mesophytes lose water very slowly.

Observations on plants in the open have shown that on bright days in early summer xerophytes of the type mentioned above reduce the water content of their leaves in the afternoon. The decrease in the water content attains, according to the determinations of Mrs. T. KRASNOSSELSKY-MAXIMOW, 30 per cent. of the water reserve of the leaves. In the hottest and dryest period the xerophytes have their leaves in a state of permanent wilting.

These observations induced Prof. MAXIMOW to study the phenomenon of wilting, and the relation to it of plants of different ecological types. It developed that xerophytes were much more enduring to wilting than mesophytes; and just in this capacity of enduring permanent wilting without injury lies the chief peculiarity of drought resisting plants.

The capacity of enduring wilting is not quite constant for each plant. It varies with the conditions of growth. Plants cultivated in moist air and in a moist soil become very sensitive to wilting. On the contrary, plants that have undergone severe wilting become considerably more tolerant of drought. This increase of drought resistance is combined with definite anatomical changes. The cells of the newly formed leaves become smaller, the number of stomata per unit area increases, and the venation becomes denser. A physiological consequence of these changes in structure is an increase of transpiration and assimilation.

Xeromorphic structure can be the consequence of dryness of the atmosphere, as well as that of the soil. Prof. ZALENSKY long ago showed that the upper leaves of a plant are always of a more xerophytic nature than the lower ones, and in accordance with this ALEXANDROV has shown that the upper leaves transpire and assimilate more intensely. Sun leaves are also more xeromorphic than shade leaves.

The xeromorphic structure reflects the changes in the physiological properties of the plant; it accompanies the increase in the osmotic pressure of the cell sap as well as the increase of the water-retaining capacity of the colloids of the plasma. This is just what makes the plants more tolerant of wilting. Besides, these anatomical peculiarities favor a better water supply when the amount of water in the soil is low, and therefore the xeromorphic plants can develop better in dry habitats.

Not all the xerophytes possess xeromorphic structure. Succulents, which are provided with a big water reserve, represent a special physiological type. Ephemeres, too, represent a peculiar type. Their anatomical peculiarities must not be considered in fixing the fundamental characteristics of a xeromorphic structure.

The widespread opinion that the bacteria and *Cyanophyceae* of hot springs may be relicts of the original plant population of the earth was combated by Dr. T. V. VOUK, of the Botanicki Zaved, Zagreb, Jugoslavia.

He bases his objections to this theory on the facts that thermophiles are phylogenetically heterogeneous and that practically all of them have close relatives widely distributed in cold fresh waters. His own experiments convince him that even the organisms inhabiting the hottest waters are still in a more or less plastic condition as regards adaptation to their peculiar environment. He believes that the present inhabitants of thermal springs migrated into the hot waters at different periods of the earth's history, though at this time it is impossible to say when, because as yet we have no facts whatever on which to base conclusions.

The difficulties of propagating plants from seeds and cuttings were discussed in a very illuminating paper by Dr. WILLIAM CROCKER, who gave an account of the work being done along this line at the Boyce Thompson Institute. Methods of stratification of seeds at 5° C., and of producing rooted cuttings of plants that seldom root, were described. Important progress has been made toward inducing rooting of cuttings of many kinds of woody plants.

The problem of the germination of orchid seeds and its relation to the supposed symbiotic fungi of orchids was discussed at length by Dr. LEWIS KNUDSON, of Cornell University. KNUDSON has shown that the orchid seedlings apparently begin life as saprophytes, dependent upon the fungus to hydrolyze the starch of the natural or the cultural substrate into glucose. Seeds provided with nutrient salts alone, or with salts plus starch, will not germinate, but if provided with the proper fungus on the starch, or with a glucose substrate and no fungus, they will thrive perfectly well. The glucose relation seems to be an obligate one, for a similar molecular concentration of other sugars fails to promote growth. The fungus relation, though obligate at first in the absence of glucose, is unnecessary later; for sterile sugar-fed seedlings were later transferred to sugarless uninoculated media and continued to grow. The symbiosis is not specific, for germination was induced by fungi other than the true orchid fungus. The fungus, a friend at first, is apparently at bottom an enemy; for it acts as a pathogen if the food supply is low, and may even prevent germination altogether. Pathogenicity seems to be specific in some instances, for fungus isolated from *Cattleya* and *Epipactis* killed seed of *Odontoglossum*.

There is no evidence yet presented that the fungus is of value unless the constancy of the association of fungus and orchid be assumed as evidence of a significant relationship. The inherent characteristics of the seed which make germination slow and uncertain must be ascribed to the lack of reserve foods and the purely saprophytic character of the embryos during the critical periods of growth.

The last two papers were presented by Dr. WALTER T. SWINGLE, of the U. S. Department of Agriculture, with ROY W. NIXON as joint author in

one of them. According to Mr. SWINGLE, experiments that have been under way for the past three years at the U. S. Date Experiment Station at Indio, California, and at other points in California and Arizona, have demonstrated very strikingly the influence of pollen, not only on the size, shape and character of the seed, but also on the size, shape and character of the fruit of the date palm, and what is still more striking, on the time of ripening. It seems impossible to reconcile the observed effects of different kinds of date pollen with the existing theory of the influence of the male gamete. The double fertilization of the egg cell and the endosperm mother cell explains satisfactorily the influence of the pollen on the date seed itself, this being a form of xenia. However, the striking influence on the size, shape, character, and time of ripening of fruit cannot be explained by the theory of xenia, but apparently necessitates the recognition of influences in the plant body analogous to those exerted by ductless glands in the animal body. The simplest hypothesis is that the embryo, or the endosperm of the date seed, or both together, constitute a ductless gland apparatus that affects, by secreting hormones, the whole development of the date fruit, including tissues belonging morphologically to the mother plant, and especially to the thickened ovary walls which constitute the edible portion of the date fruit.

Similar effects have been noted also in citrus fruits. The phenomenon is called meta-xenia.

In addition to the sectional and joint programs, there were two lectures of general interest by prominent physiologists. On Wednesday afternoon Prof. F. E. LLOYD, president of the American Society of Plant Physiologists, presented his wonderful moving picture account of the contractile vacuole of *Spirogyra*. And one of the two general evening programs was devoted to a lecture by Prof. F. A. F. C. WENT, of Utrecht, on plant movement. He discussed particularly the work done in his laboratory on the physiology of phototropism. He and his son have uncovered convincing evidence for the existence of a photolabile growth-controlling substance in dark-grown seedlings. Severed tips of *Avena* coleoptiles, offset to opposite sides of their decapitated stocks after irradiation, induced a bending *away* from the light, and always in the direction determined by the contact of the severed tip. Similar tips placed on decapitated plants of other species, as for instance stimulated oats tips transferred to unstimulated barley stocks, produced like results. And finally, the same results followed the application of blocks of gelatin on which the stimulated tips have been allowed to stand for a time, and into which the hypothetical growth-controlling substance had presumably diffused.

But the interest of plant physiologists was not limited to what went on within the four walls of their own auditorium, or the rooms in which they

held their round tables and joint meetings with other sections. There were a number of papers in other sections that now and again lured them afield. Space limitations prohibit discussion of all of these, but mention of a few may well find a place.

Dr. WILLIAM SEIFRIZ, of the University of Pennsylvania, presented some of the results of his work on the structure of protoplasm. By means of magnetic fields applied to bits of nickel and cobalt introduced into cells by means of most ingenious and skilful micromanipulations, he has been able to gain some new information on the elasticity of their living contents. His results lead him to hold for a fibrous structure of protoplasm, as against the alveolar and other theories.

Prof. W. SZAFER, of the University of Krakau, presented a new successional series from the peat bogs of Poland, giving evidence of the climate of that part of Europe during the last interglacial period. His paper was supplemented in a way by Prof. H. C. COWLES, who discussed the succession point of view in floristics. The whole succession program of the section for ecology, participated in by W. H. PEARSALL, W. S. COOPER, G. E. DURIETZ and A. G. TANSLEY, held many points of interest for physiologists, as did also some of the forestry papers, notably those on root habits of tree seedlings, and the influence of light and shade. Mention should also be made of the gene symposium on Thursday night, participated in by R. A. EMERSON, E. M. EAST, HERMAN J. MULLER and WILLIAM H. EYSTER, who presented the gene as the basis of life, and controller of variation and heredity.

A part of the discussions in the sections for bacteriology, mycology, and pathology were to be devoted to the questions of the validity of taxonomic groupings based on physiological specificities where no morphological differences are evident. In tangled questions of this sort, obviously no definite conclusions can be expected at once; but it was felt that progress had been made toward a mutual understanding, and that satisfactory legislation on the subject may some day be looked for.

On Friday afternoon the physiologists met with the bacteriologists to hear a discussion of oxidation-reduction potentials and their measurement, by WILLIAM MANSFIELD CLARK, of the U. S. Hygienic Laboratory, Washington. It was a technical, and incisively critical discussion of the previous work on measurements of these potentials in biological materials. There is, however, some hope that indicators may be found which will make such measurements as practical as measurements of hydrogen ion concentration. Most of the past work is entirely untrustworthy according to Dr. CLARK, and will have to be repeated with more accurate methods when they have been developed.

Physiologists are usually willing to admit that discussions in sections for systematic botany go completely over their heads; but at Ithaca there were several things in the taxonomy section very much worth hearing, especially the papers on plant distribution. Notable among these were those of A. W. HILL, of Kew, on the Antarctica problem, of Prof. M. L. FERNALD, of Harvard University, on the post glacial flora of the northern hemisphere, and of Dr. C. H. OSTENFELD on the flora of the contemporary glacial period in Greenland.

A report of the Congress would be incomplete without mention of the social features, and general arrangements for the meeting. The physiologists held their dinner Wednesday evening in Prudence Risley Hall, and it was well attended. After dinner speaking was taboo, and it was made a time of informal good fellowship.

The general arrangements were almost ideal, thanks to the excellent work of the local committee on arrangements, and the program committee. Especially commendable were the efforts of Drs. L. W. SHARP, L. C. PETRY and H. H. WHETZEL, who seemed to be everywhere at once, overseeing everything and overlooking nothing that contributed to the comfort of attendants or to the success of the Congress. The registration went off smoothly and without crowding, the visitors were all quickly and satisfactorily quartered, and the ample spaces of Willard Straight Hall afforded abundant opportunity for the "visiting around" that is the real heart of a scientific gathering, as well as for the more ambitious but very comfortable social events that were staged. The programs were ready on the opening day, and abstracts in mimeograph form were distributed as soon as the sections began their sessions. The auditoriums were adequate, and in convenient proximity to each other, and the projection lanterns were in working order almost without exception. It was the general feeling of those who attended the Congress that it had been the best arranged and most profitable botanical meeting ever held in the United States. As an example of smooth functioning, the meeting was a joy, not only to the physiologists, but to all who were fortunate enough to be in attendance.

At the close of the Congress it was decided to hold the next International Botanical Congress at London, England, in 1930. Every one should plan if possible to attend the Fifth International Botanical Congress at that time.

SCIENCE SERVICE,
WASHINGTON, D. C.

TEMPERATURE EFFECTS IN THE METABOLISM OF WHEAT*

W. E. TOTTINGHAM[1]

(WITH THREE FIGURES)

Introduction

Early observations of environmental effects in plant growth disclosed certain relations between temperature and rate of elongation. In recent decades attention has been turned to the influence of temperature upon the rates of specific reactions in the organism and upon the gross composition of plant tissues. Behind such investigations has been recognition that modifications of metabolism and composition may be of special significance in affecting food quality, disease resistance and fruitfulness. Development of technique for determining the specific effects upon plant metabolism of individual climatic factors is relatively recent. As examples, one may refer to the climatic chambers of HOTTES (**8**), the Wisconsin soil temperature tanks (**9**) and the installation of controlled greenhouses at the Boyce-Thompson Institute (**3**). The writer (**17**) has previously published results in this field.

Two main courses of procedure become immediately apparent in approaching the problem of temperature effects in metabolism. On the one hand, one may examine the composition of organs and tissues, on the other, he may compare the rate of reaction in isolated systems, such as specific enzymatic changes. In either event, limiting values of other environmental factors than temperature must be avoided, as pointed out by BLACKMAN (**1**). In view of the lack of adequately controlled experiments in which compositional responses of the plant to temperature have been determined, we have proceeded on this course, with the recognition, however, that the two courses are mutually dependent and complementary.

Apparatus and methods

CLIMATIC CHAMBERS

For use in the present work the culture chambers were much improved from their earlier form. They were identically exposed to sunlight in

* These investigations were supported by grants from the University research funds. Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[1] With the assistance of E. J. RANKIN, A. D. DICKSON and H. W. LOUWSMA. Credit is due certain of the writer's colleagues for advice and assistance freely given, especially to Dr. J. G. DICKSON for aid in operating the chambers, and ERIC MILLER for access to special records of the U. S. Weather Bureau.

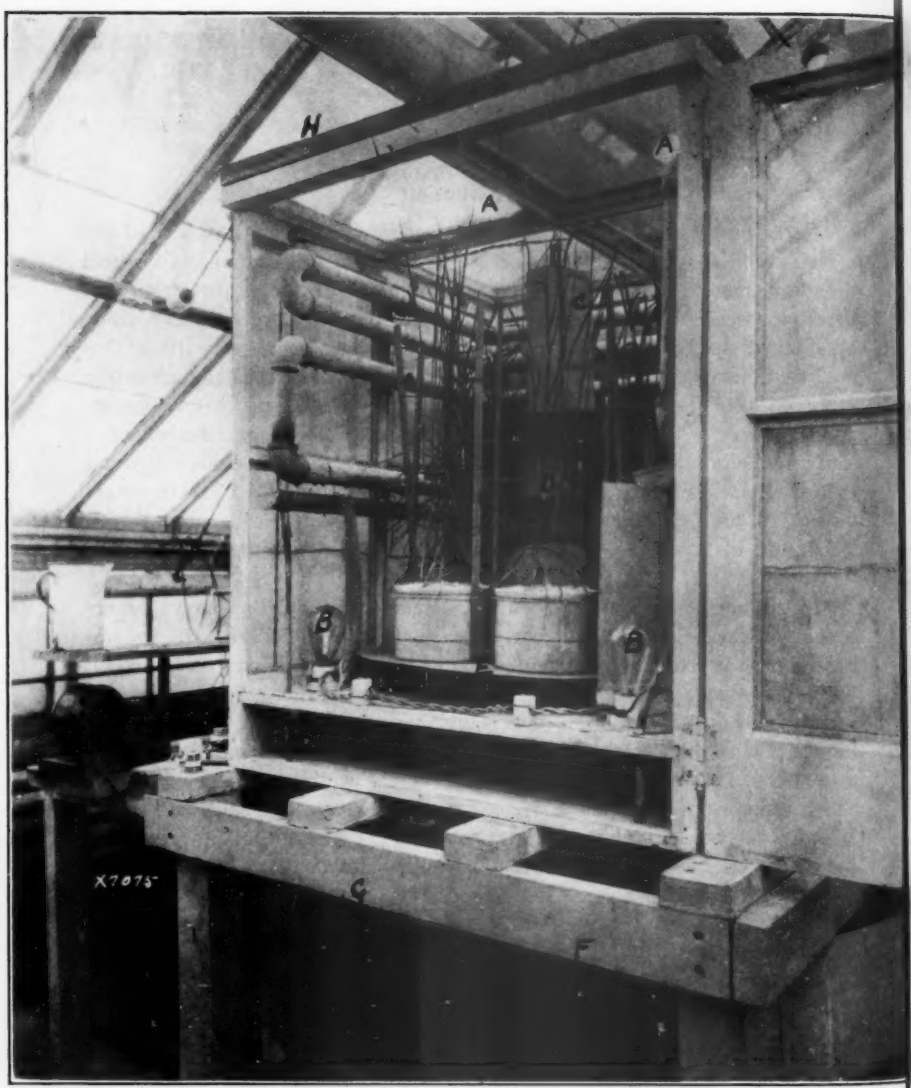

FIG. 1. Arrangement of culture chambers used. A. Illuminating lamps. B. Sockets of cylindrical heaters. C. Thermostats and humidostats, shaded. D. Hygrothermograph. E. Rotating table. F. Location of humidifier, door removed. G. Location of blower. H. Water cell.

separate compartments of a greenhouse range and attached to refrigeration service. One of these compartments was regulated roughly to a temperature of about 20° C, the other about 24° C. Chilling was effected by about 9.0 m. (30 ft.) of 3.2 cm. (1.25 in.) brine pipe supported in coils on the sides of the upper part of the chamber. By means of valves and by-passes the effective brine flow was reduced to about 3.0 m. at the higher temperature. Heaters in the form of luminous radiator units of 250 watts capacity each, erected on the floor of the culture chamber at the corners, operated against the brine coils. These functioned intermittently under the control of a bimetallic thermostat suspended at the top-center of the chamber.

The humidifying system was altered by removing the toweling, transferring the exit to the end of the tank and erecting adjacent thereto a spray nozzle which played into the approaching air current. Separate lines of hot and cold water were merged to give some control over the temperature of the mist delivered. To conserve space the humidifier was placed beneath the chamber with its exit connected upward thereto. At its entrance was fixed an electrically operated blower.[2] At first the latter was operated intermittently through a humidostat, but this proved too much of a tax upon the motor. In later tests the blower has operated continuously while its service is cut in or out by a damper. The latter is closed by a solenoid operated through the humidostat.[3] This latter control instrument[4] is a wooden cylinder so suspended as to effect a contact after the manner of a thermostat, and sensitive to small changes in relative humidity.

Previously experienced difficulty in warping of the rotating tables, which bear the plant cultures, was avoided by constructing them wholly from iron. The general arrangements of the culture chambers are to be seen in fig. 1, while the degree of control over temperature and humidity appears in fig. 2.

Illumination

In the particular study here attempted it is obviously important that illumination be kept above the plane of a limiting factor in growth. In this respect the results should be subjected to critical valuation.

Inasmuch as tests early showed the loss of light from the north side of the culture chambers this sash was closed by a sheet of asbestos. Shades were omitted from the greenhouse roof, but muslin curtains were fixed on the vertical sash of the chambers, so as to slide horizontally. The usual

[2] Sirocco no. 0 of the American Blower Company, Detroit.

[3] Acknowledgment is due the General Electric Company for assistance in the installation of magnetic switches and solenoids adapted to this apparatus.

[4] Supplied by the Johnson Service Company, Milwaukee, Wis.

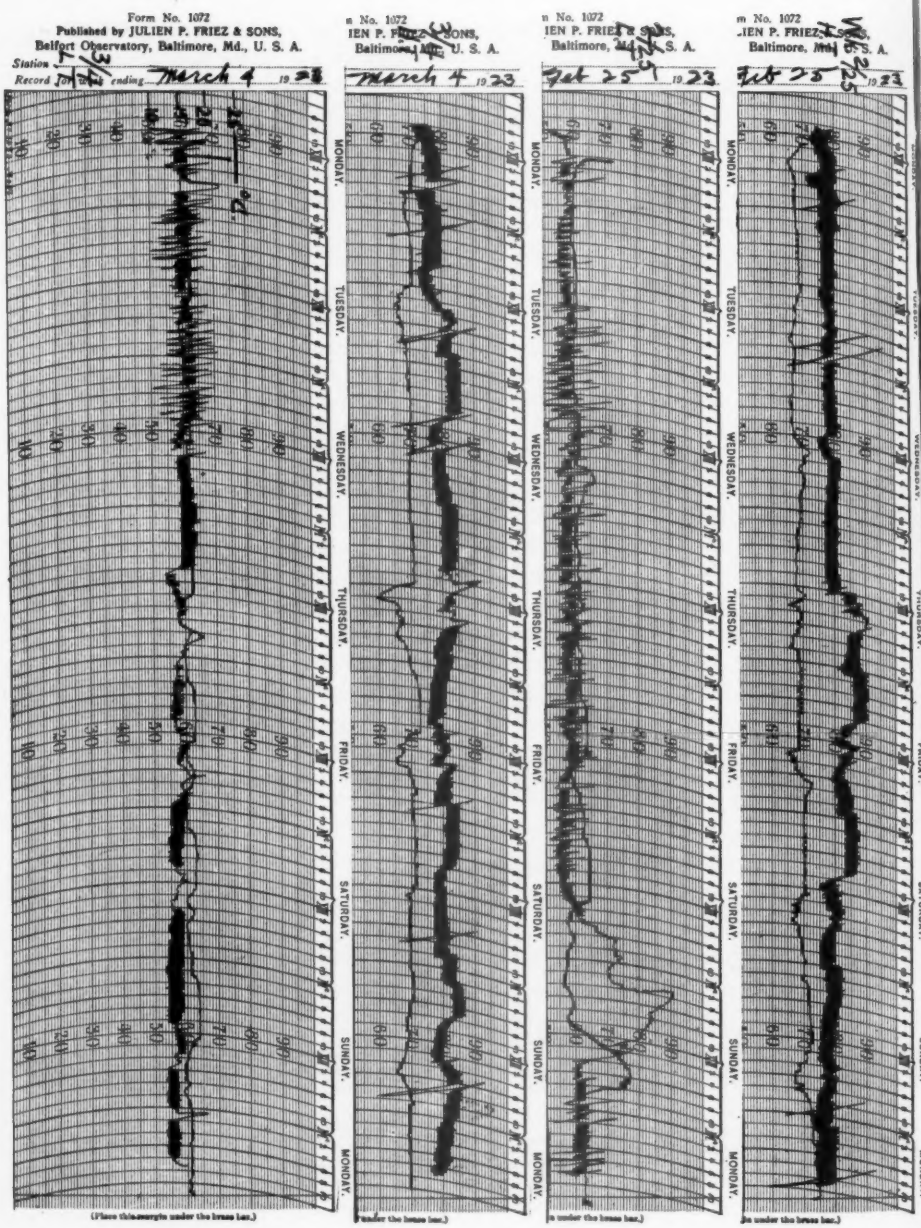

FIG. 2. Examples of control of temperature and humidity in the culture chambers.

glazing applied to the greenhouse roof has been omitted also, in the more recent tests. The artificial illumination was increased in successive tests from one 500 watt Mazda lamp to three lamps of a total capacity of 2,500 watts, which consumed the maximum current available for each chamber. In order to derive maximum illumination, by dropping the lamps close to the chambers, it was necessary to provide for elimination of excessive heat radiation. For this purpose shallow copper trays were placed upon the chambers,[5] after removing the upper panes of the top sash. These were of the same dimensions as the chamber top and bore panes of glass overlying those of the sash. The necessary plumbing attachments provided for a sheet of water flowing across this cell, the maximum depth of water being set at about 1.0 cm. With this attachment, satisfactory control over temperature was maintained except during sunny middays of early fall and late spring.

With the use of daylight it is obvious that both quality and intensity of illumination varied widely. However, these variations must have been closely parallel in the two culture chambers. It is possible to state the approximate intensities effective. Using a Sharp-Millar photometer, with a selective filter solution of both chromate and di-chromate of potassium, the intensity in the shaded chambers on a cloudy day was found to be about 0.2 that out of doors. The out-door values of sunlight have been derived from the pyrheliometric measurements of the local station of the U. S. Weather Bureau, made with a Callendar recorder. In converting these to foot-candle equivalents, data of KIMBALL have been employed. He compared measurements by a carefully standardized Sharp-Millar photometer with the simultaneous records of the pyrheliometer at Mt. Weather, Virginia, late in January. These data give an average equivalent of 139 foot candles for 1 calorie per sq. cm. per hour. Other comparisons of his photometric measurements on several dates from mid-October to mid-May, the period of operation of our chambers, with the corresponding pyrheliometric values show considerable variability in the course of seasons, but with an average of 145 foot candles per hour-calorie. We have adopted the conservative value of 140 foot candles as equivalent to 1 hour-calorie of solar radiation. Assuming the same proportion of illuminating value in the radiation admitted by the chambers, the intensity of the latter would be 28 foot candles per hour-calorie of solar radiation out of doors. Direct measurements of illumination delivered by the lamps overhead were made with the Sharp-Millar instrument. The mean hourly maximum radiation has been derived from the corresponding values in records of the local station of the Weather Bureau, making adjustment for the proportions of monthly periods involved in each culture series.

[5] Upon the advice of Dr. M. LUCKIESCH, Nela Research Laboratories.

On the basis of the relations just stated, the illumination effects in our chambers appear to have been about as follows: Efficiency of reflectors, 150 per cent.; maximum illumination from reflectored 1,000 watt lamp at soil surface 150 foot candles, at half depth of chamber 350 foot candles; range of mean maximal solar radiation, 590 foot candles in December to 1,800 in May,[6] with shades closed. Depending upon the degree of artificial illumination, this would place the mean maximal total illumination of the upper leaf surface of wheat when half grown at about 750 to 1,950 foot candles in our earliest tests and 1,450 to 2,650 in the latest ones, with the chambers shaded. In the later tests more sunlight was admitted to the chambers, at some expense of temperature control, and the mean maximal light intensities must have been considerably higher than those just stated.

There are small grounds for comparing these values with plant requirements. More accurate measurements of light intensity and determinations of the demand at different growth phases are much needed. According to HENDRICKS and HARVEY (**6**), wheat grown at 14° C. under continuous illumination by Mazda lamps requires 185 to 225 foot candles for seed production, while buckwheat at 25° C. requires about one half this intensity. It appears possible that the requirements in this respect may vary with the stage of growth. However, if we assume that the minimum intensity required varied inversely as the time of exposure, the results of HENDRICKS and HARVEY indicate a light requirement of wheat approaching 450 foot candles for a 12 hour day. This would correspond to a diurnal maximum of about 845 foot candles. If one neglects the matter of light quality, it appears that our earlier wheat cultures suffered from insufficient illumination. The adequacy of the light factor will be treated more fully in the general discussion.

NUTRIENT SOLUTION

To provide for separate variations in supply of some of the more essential nutrient elements the formulae of table I were used.

This solution provides for the relatively high requirements of young wheat plants for both potassium and nitrogen (**5**) and also permits separate varying of the supply of these elements. The use of sodium chloride throughout compensates somewhat the reduction in concentration of sodium and chlorine as the contents of nitrogen and potassium are altered. This formula is designated by the term "full." With the supply of the $NaNO_3$ reduced to one half and one fifth the full amount, the designations "0.5N" and "0.2N," respectively, are applied. The corresponding variations of potassium are designated as "0.5K" and "0.2K."

[6] The actual maxima exceeded these values by about 600 foot candles.

TABLE I

FORMULAE OF SALTS FOR A SOLUTION PERMITTING SEPARATE VARYING OF THE POTASSIUM AND NITRATE CARRIERS

FORMULAE OF SALTS USED	CONCENTRATION OF ANHYDROUS SALTS
	Per cent.
$MgSO_4.7H_2O$	0.025
$Ca(H_2PO_4)_2.H_2O$	0.040
NaCl	0.025
KCl	0.075
$NaNO_3$	0.075
Ferric citrate	0.001

Cultural treatment and results

Throughout the present work pure line seed of the Marquis variety of wheat has been used, selected for uniformity of size from stock not over two years old. Unless stated otherwise, temperature control began with the germination phase of growth. Details of the procedure for water culture will be found elsewhere (**16**). The climatic records are given for the period of simultaneous growth at the two temperature ranges in each test. These were taken in part on hygro-thermographs, but the adjustments for humidity control were directed toward equalizing the evaporation from atmometers carried on the rotating tables. Unless specified otherwise, the plants were killed promptly after harvesting by heating about 30 minutes at 98° C. in a closed oven. Subsequent desiccation occurred at about 70° C. with ventilation. The roots were severed from the tops to prevent translocation of materials during drying.

WATER CULTURES

SERIES 1.

Seedlings selected from a large group were set up in triplicate cultures of five plants each for each variation of the cultural treatment. At the higher temperature this transfer occurred seven days after placing the soaked seeds upon the germination net. These seedlings were then about 10 cm. tall and three times as tall as those grown at the lower temperature, which were set up two days later. The culture vessels had a capacity of 960 cc. (quart Mason jars) and the solutions were renewed twice a week. Throughout the test the full concentration of nutrients was employed but during germination the P_H was adjusted to about 7. At the higher temperature the roots early developed an abnormal mucous coating, while the

advent of withering in the tops of these cultures marked the termination of the test. As a result of this experience the maximal temperature in later series was advantageously reduced.

This series grew from December 1, 1922, to January 10, 1923, including the germination periods in the respective chambers. The following climatic conditions prevailed in the chambers: Temperature about 25° and 15° C.; relative humidities about 70 and 50 per cent.; maximum artificial radiation 175 foot candles; total solar radiation 970 calories; mean hourly maximum of solar radiation 650 foot candles; mean hourly solar radiation (eight hour day) 410 foot candles; evaporation from the standard black atmometer 559 cc. at the higher temperature, and 517 cc. at the lower, or an excess of 8 per cent. at the higher temperature.

Linear measurements of the plants were rather uniform within each temperature group, but the tops were about 40 per cent. taller at the lower temperature. The yield and composition of tops are given in table II. In

TABLE II

COMPOSITION OF DRY TOPS OF SERIES 1. GROWTH PERIOD 41 DAYS. AVERAGE TOTAL ILLUMINATION IN 8-HOUR DAY, 580 FOOT CANDLES

TEMPERATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER[a]	ETHER EXTRACT	POLYSACCHARIDES[b]	CRUDE PROTEIN[c]	YIELD OF CRUDE PROTEIN
° C.		Cgm.	Per cent.	Per cent.	Per cent.	mg.
25	Full	54	6.6	7.1	7.1	44
25	0.5N	54	8.7	9.7	6.9	37
25	0.2N	54	11.4	8.9	6.9	37
25	0.5K	54	7.1	5.2	7.1	38
25	0.2K	48	7.0	9.1	7.1	34
15	Full	84	11.5	12.1	7.1	60
15	0.5N	87	10.2	10.3	5.7	50
15	0.2N	78	10.7	9.8	5.7	44
15	0.5K	81	10.5	12.3	7.8	63
15	0.2K	84	9.6	10.0	5.8	49

[a] Total of 3 cultures, or 15 plants.

[b] Hydrolysis of sugar-free tissue for 1 hour with 3.7 per cent. H_2SO_4.

[c] $N \times 6.25$ in residue from acid hydrolysis.

view of the limited growth these merit little discussion. It appears probable that the illumination was considerably deficient. The yield was greater at the lower temperature by 60 per cent. Likewise, the contents of ether extract and polysaccharides were generally favored by the lower temperature.

SERIES 2.

Twenty-five seedlings left by selection from about 200 were transferred on each germination net to a battery jar of 3.5 liters capacity. This was coated with asphaltum paint to exclude light. The plane of nutrients was increased by steps from 0.2 full to full concentration, while a P_H value of about 7 was maintained throughout the test. In the final week the solutions were not renewed, so as to accentuate the relative absorption of nutrients. Drying of samples for analysis was here conducted throughout at about 55° C. As a special phase of the analytical examination of the plant tissues, determinations were made of the proportion of pentoses present, by fermentation of the hexoses with a pure strain of yeast.[7]

This series grew from February 18 to March 30, 1923, being terminated only by limitation of time. The following climatic conditions prevailed: Temperature planes about 23° C. and 13° C., with relative humidities of about 80 per cent. and 60 per cent. respectively; maximum artificial radiation at mid-chamber 175 foot candles; total solar radiation 3,040 calories; mean hourly maximum of solar radiation 1,310 foot candles; mean hourly solar radiation (basis of 10 hour day) 1,040 foot candles; total evaporation from the standard black atmometer 616 cc. at the higher temperature and 516 cc. at the lower or 19 per cent. greater in the former case. Solar radiation effective during the test of absorption of nutrients averaged 95 calories per day, equivalent to a maximum of about 2,000 foot candles, and an average total illumination of 10 hour day, 1,500 foot candles (1,720 foot candles on day of harvesting).

The only significant variation in linear measurements was an average elongation of the tops at the higher temperature of about 40 per cent. Both tops and roots were considerably greater in diameter at the lower temperature. Tables III, IV and V show the yield, composition and absorption values.

With due allowance for difference in numbers of plants involved, the production of dry matter was much more extensive in this series than in the previous one. This difference is greater than can be accounted for by the difference in intensity of solar radiation for the growth periods. The yield and composition of tops were much the same for the two planes of temperature. Only the insoluble protein was consistently higher in the plants grown at the lower temperature. The pentose content of the hemicellulose was somewhat lower also in this case. The roots were a little heavier at the lower temperature than the higher one and contained, as an average, over 50 per cent. more "available" carbohydrates in the former case. On a reduced supply of nitrate, protein synthesis appears to have been hindered

[7] Under the direction of Dr. E. B. FRED, bacteriologist of this institution.

TABLE III

COMPOSITION OF DRY TOPS OF SERIES 2. GROWTH PERIOD, 41 DAYS. AVERAGE TOTAL ILLUMINATION OF 10-HOUR DAY, 1,210 FOOT CANDLES. DRY WEIGHT BASIS

TEMPERATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER	ETHER EXTRACT	DEXTRINS AND STARCH	HEMICELLULOSE[a]	PENTOSE OF HEMICELLULOSE	INSOLUBLE PROTEIN	YIELD OF INSOLUBLE PROTEIN
° C.		Gm.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
23	Full	3.01	5.4	1.0	13.6	9.9	4.6	138
23	0.5N	3.33	6.7	1.0	13.7	9.9	4.5	150
23	0.2N	2.98	3.5	1.0	14.0	9.2	4.3	128
23	0.5K	3.63	6.1	0.8	13.8	8.5	4.5	163
23	0.2K	3.74	6.8	0.8	13.9	9.3	4.5	168
13	Full	2.71	7.2	0.6	9.3	6.4	6.2	162
13	0.5N	3.09	6.6	1.2	12.0	7.2	6.9	213
13	0.2N	3.17	6.8	1.3	12.9	7.2	5.6	177
13	0.5K	2.79	6.0	0.8	12.1	5.6	8.1	226
13	0.2K	2.81	7.3	0.7	12.9	8.4	6.3	177

[a] Hydrolysis for 1.0 hour with 2 per cent. H_2SO_4.

at the higher temperature and favored at the lower, but the samples were too small to give very significant results.

TABLE IV

COMPOSITION OF DRY ROOTS OF SERIES 2. GROWTH PERIOD 41 DAYS. AVERAGE TOTAL ILLUMINATION OF 10-HOUR DAY, 1,210 FOOT CANDLES. DRY WEIGHT BASIS

TEMPERATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER	SUGARS AND POLYSACCHARIDES[a]	INSOLUBLE PROTEIN	YIELD OF INSOLUBLE PROTEIN
° C.		Cgm.	Per cent.	Per cent.	mg.
23	Full	39	14.4	7.8	31
23	0.5N	41	13.1	6.4	26
23	0.2N	56	10.8	5.7	32
23	0.5K	42	11.6	7.4	31
23	0.2K	39	13.7	7.6	30
13	Full	56	20.1	6.8	38
13	0.5N	63	18.8	8.1	51
13	0.2N	54	19.2	8.3	45
13	0.5K	53	19.5	6.2	33
13	0.2K	49	20.1	6.0	29

[a] Ether extract not removed. Hydrolyzed 1 hour with 2 per cent. H_2SO_4.

The data for absorption of nutrients show increased up-take of potassium at the higher temperature and of nitrogen at the lower, on the full solution. At the lower planes of supply of potassium the results indicate a greater requirement of this element at the lower temperature. This is in accord with the results of GERICKE (**4**). An increased requirement for nitrogen at the higher temperature is indicated by the increased absorption of this element on the partial planes of supply. With the lowest supply of the

TABLE V

ABSORPTION FROM NUTRIENT SOLUTION OF SERIES 2. AVERAGE TOTAL ILLUMINATION OF TEST PERIOD IN 10-HOUR DAY, 1,500 FOOT CANDLES. EVAPORATION FROM STANDARD ATMOMETER, 57.8 CC. AT 23° C., AND 57.6 CC. AT 13° C.

ELEMENT TESTED	TEMPERATURE °C.	NUTRIENT SUPPLY				
		Full	0.5N	0.2N	0.5K	0.2K
Nitrogen	23	10[a]	56	−31[b]	8	10
Nitrogen	13	19	37	40	12	18
Potassium	23	32	21	26	15	42
Potassium	13	19	16	26	20	61

[a] Percentage of the amount presented.

[b] Recovery of 131 per cent.

element the results give evidence of a return to the last portion of solution of unassimilated nitrate, which had been absorbed from previous supplies. This negative balance of nitrogen might be expected to eventuate critically if prolonged, but the data of yields and composition give no distinct evidence of such conditions. It is possible that cloudiness of the day preceding harvesting, when an average total radiation of about 1,100 foot candles was effective, induced a change of energy relations in the plant which were responsible for the excretion of nitrate. HOAGLAND (7) has shown the importance of illumination in this regard. While much more adequate than in series 1, the illumination may have been somewhat deficient here.

SERIES 3.

This test was conducted in the same manner as the previous one, excepting the increase of plants per culture to 40. Development of seedling blight (*Helminthosporium*) occurred to some extent at the higher temperature. The seriously affected plants were eliminated in preparing the tissue for chemical analysis.

Growth here covered the period from October 24 to December 24, 1923. The climatic conditions effective in the chambers were as follows: Temperature planes, about 23° and 13° C.; maximal artificial radiation at midchamber, about 340 foot candles; total solar radiation 1,590 calories; mean hourly maximum of solar radiation, 670 foot candles; mean hourly solar radiation (basis of 8-hour day) 450 foot candles; evaporation from the standard black atmometer 524 cc. at the higher temperature and 556 cc. at the lower, or about 6 per cent. excess in the latter case. Solar radiation effective during the measured absorption of nitrate averaged 17 calories

TABLE VI

YIELD AND ABSORPTION OF NUTRIENTS IN SERIES 3. GROWTH PERIOD, 62 DAYS. AVERAGE TOTAL ILLUMINATION OF 8-HOUR DAY, 790 FOOT CANDLES

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER OF TOPS	DRY MATTER OF ROOTS	ABSORPTION OF NITRATE
	° C.	Cgm.	Cgm.	Per cent.
Full	23	73 ± 0	27 ± 2	16.8 ± 0.4
Full	13	95 ± 6	32 ± 1	14.1 ± 0.2
0.5N	23	64 ± 6	25 ± 3	−32.7 ± 4.5[a]
0.5N	13	90 ± 6	31 ± 1	−38.4 ± 2.7
0.2N	23	70 ± 4	26 ± 1	51.1 ± 2.1
0.2N	13	91 ± 0	38 ± 1	43.7 ± 10.6

[a] Negative absorption denotes increased concentration in the solution by leaching from the plants.

per day, equivalent to a maximum of about 360 foot candles, and average total illumination of 8-hour day, 630 foot candles (950 foot candles on day of harvesting).

TABLE VII

COMPOSITION OF DRY TOPS IN SERIES 3

NUTRIENT SUPPLY	TEMPERATURE	ETHER EXTRACT	REDUCING SUGARS	POLYSACCHARIDES[a]	INSOLUBLE PROTEINS[b]	YIELD OF INSOLUBLE PROTEIN
	° C.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
Full	23	6.8 ± 1.0	1.6 ± 0.2	12.8 ± 1.7	2.9 ± 0.4	21
Full	13	8.1 ± 1.0	0.5 ± 0.1	7.3 ± 0.3	5.2 ± 0.9	49
0.5N	23	9.2 ± 1.8	1.1 ± 0.9	8.0 ± 0.9	3.5 ± 0.2	22
0.5N	13	9.1 ± 1.8	0.4 ± 0.1	7.2 ± 0.4	10.7 ± 0.0	96
0.2N	23	6.5 ± 0.3	0.5 ± 0.0	3.7 ± 1.6	3.3 ± 0.1	23
0.2N	13	9.4 ± 0.5	0.3 ± 0.0	3.6 ± 0.1	9.3 ± 0.3	85

[a] Hydrolysis 1 hour with 2 per cent. H_2SO_4.

[b] N × 6.25 in residue from acid hydrolysis.

The results of this series appear in tables VI, VII and VIII. Both tops and roots gave an average increase of about 30 per cent. in dry weight at the lower temperature. The only differences in chemical composition which appear significant occurred in the tops. Here the polysaccharide content was favored by the higher temperature on the full nutrient solution, while insoluble protein was most abundant throughout at the lower temperature. Reducing sugars were appreciably more plentiful in the tops at the higher

TABLE VIII

COMPOSITION OF DRY ROOTS OF SERIES 3

NUTRIENT SUPPLY	TEMPERATURE	ETHER EXTRACT	REDUCING SUGARS	POLYSACCHARIDES[a]	INSOLUBLE[b] PROTEIN	YIELD OF INSOLUBLE PROTEIN
	° C.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
Full	23	6.8	0.5	21.4	5.1	14
Full	13	4.7	0.5	19.3	3.4	11
0.5N	23	5.4	0.5	20.7	3.7	9
0.5N	13	4.8	0.5	26.8	2.7	8
0.2N	23	5.0	0.5	20.3	4.7	12
0.2N	13	4.4	0.6	19.2	2.9	11

[a] Hydrolysis 1 hour with 2 per cent. H_2SO_4.

[b] N × 6.25 in residue from acid hydrolysis.

temperature and the same applies to insoluble protein in the roots. A negative balance of nitrate occurred in all cultures at the 0.5N concentration. It appears most probable that the difference from series 2, where this effect was found in the 0.2N solution at the higher temperature, should be ascribed to the differences in illumination. Apparently the plant is quite sensitive to this factor in the aerial environment. The average light intensity of this test was much less adequate than in the preceding one and obviously deficient, as indicated by the yields.

Sand cultures

Series 4.

Sand of high purity was used,[8] composed of spherical particles grading 60 to 70 mesh. Portions of 3.2 kgm. each were used in stoneware jars of 1.9 liters (2 quart) capacity. The water plane was increased by three steps from 25 per cent. of the holding capacity at planting to 50 per cent. at active growth, and reduced later to favor maturation of the plants. Nutrient salts were added in solution in three successive increasing portions. The total amounts per culture for the full solution were as follows: $NaNO_3$ 1.0 gram, equi-molar mixture of $Na_2HPO_4.10H_2O$ and $NaH_2PO_4.H_2O$ 0.15 gram, KCl 0.2 gram, $CaSO_4.2H_2O$ 0.1 gram, $MgSO_4.7H_2O$ 0.1 gram and ferric citrate 0.04 gram. Surface sterilization for the suppression of seedling blight (*Helminthosporium*) was effected by the use of semesan, an organic mercurial preparation. Fourteen seedlings per culture were left

TABLE IX

Yield and composition of straw in series 4. Growth period, 80 days. Average total illumination of 12-hour day, 1,150 foot candles. Dry weight basis

Nutrient supply	Temperature	Yield of dry matter	Nitrate radicle	Soluble protein[a]	Insoluble protein[b]	Yield of insoluble protein
	° C.	Gm.	Per cent.	Per cent.	Per cent.	mg.
Full	21–22	1.43 ± 0.33	2.3	4.4	8.7	124
Full	16–17	4.63 ± 0.23	0.5	3.8	8.7	403
0.5N	21–22	1.73 ± 0.13	1.1	5.6	8.1	149
0.5N	16–17	4.00 ± 0.10	0.2	3.1	8.1	344
0.2N	21–22	1.38 ± 0.03	0.6	3.1	8.7	120
0.2N	16–17	3.30 ± 0.25	0.2	1.9	6.3	208

[a] Non-nitrate N × 6.25 in water extract.

[b] N × 6.25 in residue from watery extraction.

[8] Supplied by Ottawa Silica Company, Ottawa, Illinois.

by selection from three times that number. After two months of growth the plants subjected to the lower temperature were equal in height to contemporaneous greenhouse cultures on soil, while those at the higher temperature were considerably shorter and characteristically spindling. Despite hand pollination from plants blooming in the greenhouse only two or three shriveled seeds were produced, and these at the higher temperature. This result may have been due to unfavorable effects of the temperatures upon pollen growth, as well as to cumulative effects upon the plant as a whole. Heads are included in the straw weights but they were discarded in preparing the tissue for analysis. The analytical data appear in table IX.

Growth after emergence from the soil occurred from March 4 to May 19, 1924, in the warmer case and four days later in the colder one. The climatic data for this series were as follows: Temperature planes 16–17° C. and 21–22° C.; maximal artificial radiation at mid-chamber 340 foot candles; total solar radiation 5,320 calories; mean hourly maximum of solar radiation 1,300 foot candles; mean hourly solar radiation (basis of 12-hour day) 810 foot candles; evaporation from the standard black atmometer 615 cc. at the lower temperature and 672 cc. at the higher. Thus the evaporating power was about 9 per cent. greater at the higher temperature.

The yield was nearly 150 per cent. greater at the lower temperature and here also production increased with the nitrate supply. At the higher temperature the total nitrogen content of the tissue was higher, which is practically equivalent to stating that carbohydrates were most abundant at the lower temperature. It increased with the plane of supply, most distinctly at the lower temperature, the variations occurring in soluble forms. On the higher planes of supply the nitrate content of the tissue at the higher temperature was more than inversely proportional to yield, indicating a greater capacity for assimilating this radicle at the lower temperature.

SERIES 5.

This series of wheat cultures was conducted in the same manner as the previous one with the exception of a portion of the environmental exposure. When it appeared that the blossoms were formed and pollination was approaching, the cultures were gradually removed to a greenhouse exposure of about 20° C. until seeds were formed. They were then gradually subjected to the original temperatures of their respective chambers. This removal period extended from April 13 to April 29 at the higher temperature and April 28 to May 14 at the lower one, the former interval corresponding to 3,130 calories of solar radiation effective in the house, the latter to 3,750. In tables X and XI are assembled the data of yields and composition.

TABLE X

YIELD AND COMPOSITION OF GRAIN IN SERIES 5. GROWTH PERIOD, 99 DAYS. AVERAGE TOTAL ILLUMINATION OF 12-HOUR DAY, 1,560 FOOT CANDLES

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER	AVERAGE WEIGHT PER SEED	PROTEIN CONTENT[a]	TOTAL YIELD OF PROTEIN
	° C.	mg.	mg.	Per cent.	mg.
Full	18–20	87 ± 40	10.5 ± 1.5	27.5	48
Full	12–14	214 ± 112	13.5 ± 0.5	28.7	123
0.5N	18–20	130 ± 43	14.0 ± 2.0	24.4	63
0.5N	12–14	458 ± 108	16.5 ± 1.5	23.4	214
0.2N	18–20	65 ± 21	25.5 ± 3.5	19.3	25
0.2N	12–14	400 ± 123	18.5 ± 2.5	18.1	145

[a] Total N × 6.25.

This series grew in the period February 18 to May 27, 1925, at the higher temperature, extending to June 10 at the lower one. The climatic data follow: Temperature ranges, 13–15° C. and 18–20° C.; relative humidity, about 55 and 75 per cent. respectively; maximum artificial radiation, about 500 foot candles; total solar radiation effective in house or chamber, 9,560 and 9,940 calories at the higher and lower temperatures respectively; mean hourly maximum of solar radiation, 1,740 and 1,810 foot candles respectively; mean hourly radiation of 12-hour day, at high temperature 900 foot candles in chamber, 2,280 in house, average 1,100; at low temperature 870 foot candles in chamber, 2,730 in house, average 1,140 foot candles; evaporation from the standard black atmometer 587 cc. at the lower temperature

TABLE XI

YIELD AND COMPOSITION OF STRAW IN SERIES 5

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER	CRUDE PROTEIN CONTENT[a]	CONTENT OF NITROGEN BASES[b]	TOTAL YIELD OF PROTEIN[c]
	° C.	Gm.	Per cent.	Per cent.	mg.
Full	18–20	2.41 ± 0.14	12.9	5.9	284
Full	12–14	4.44 ± 0.04	10.9	6.7	596
0.5N	18–20	2.32 ± 0.29	11.1	4.8	223
0.5N	12–14	3.69 ± 0.26	7.7	3.9	288
0.2N	18–20	1.44 ± 0.58	13.4	6.6	190
0.2N	12–14	3.20 ± 0.02	4.8	2.8	179

[a] Total N × 6.25.

[b] N precipitated by phosphotungstic acid × 6.25.

[c] Dry matter × nitrogenous bases.

and 636 cc. at the higher one. Thus the evaporation was about 8 per cent. higher at the higher temperature.

It appears that the intensity of illumination could hardly have been seriously deficient for these cultures. The data of total yields show 60 to 120 per cent. increases in development of straw at the lower temperature and a corresponding increase of 150 to 500 per cent. in the grain. Despite these wide variations in growth the crude protein content of the grain differed little at the two temperatures. This seems not to hold for the straw. The plane of nitrate supply was more effective than temperature difference in affecting the content of protein of the seed. In the case of the straw, however, this response occurred only at the lower temperature.

Computation shows that 43 to 49 per cent. of the total nitrogen in straw of the high temperature cultures was precipitable by phosphotungstic acid, as compared with 51 to 62 per cent. at the lower one. The difference was most marked with the full nutrients, and is very likely due to inferior assimilation of nitrate at the higher temperature, as observed in series 4.

Soil cultures

Series 6.

In order to correct still further the possible abnormal conditions of the environment, this series was grown on Miami silt loam to which a small application of complete fertilizer was added. Each 2-quart stoneware jar supported 15 plants. Optimal planes of soil moisture were maintained to maturity. The cultures progressed in a greenhouse, at temperatures ranging from 15° C. (60° F.) by night to 20° C. (70° F.) by day, until the seeds had formed. During the remainder of development they were divided into two lots, one lot being gradually adjusted to the conditions of each climatic chamber. The data of yields and composition appear in table XII.

TABLE XII

Yield and composition of series 6. Growth period, 82 days. Average total illumination of 13-hour day, 2,320 foot candles

Temperature	Yield of dry straw[a]	Protein content of straw[b]	Yield of dry grain[a]	Average weight per seed	Protein content of grain[a]	Average yield of protein
° C.	Gm.	Per cent.	Gm.	mg.	Per cent.	Cgm.
18–20	9.3 ± 1.3	3.1 ± 0.7	2.3 ± 1.0	11 ± 3	19.4 ± 5.0	73
12–14	10.4 ± 2.2	2.4 ± 0.1	3.5 ± 1.9	15 ± 6	15.6 ± 1.6	80

[a] Average of 6 cultures ± average maximum departure.

[b] Average of 3 cultures ± average maximum departure, determined by precipitation with phosphotungstic acid.

The total growth period after germination extended from March 24 to June 13, 1925, at the higher temperature and to June 17 in the other case, with the transfer to the chambers occurring on May 27. Climatic records for the entire growth period follow: Temperature ranges 13–15° C. and 18–20° C.; maximum artificial radiation about 500 foot candles; total solar radiation, 14,740 calories in the house and 2,010 calories in the chambers; mean hourly maximum of solar radiation, 3,490 foot candles; mean hourly solar radiation, 2,480 foot candles in house (basis of 13-hour day), 1,120 foot candles in chamber (basis of 14-hour day); evaporation during the control period from the standard white atmometer, 126 cc. at the higher temperature, and 116 cc. at the lower one. Thus the evaporation was about 9 per cent. greater at the higher temperature.

The yields of grain at the lower temperature averaged 35 per cent. greater than at the higher one, while there was a lesser difference of 10 per cent. in the case of the straw. This temperature effect is more marked if one eliminates the values of one immature culture at the lower temperature. The weight of individual seeds averaged 36 per cent. higher at the lower temperature. As would be expected, this was associated with increased starchiness at the lower temperature. In terms of the protein content the seeds produced at the higher temperature were richer by 4 per cent., based upon the dry matter. The seeds reared at the lower temperature had the same protein content as the grain sown. Based upon this value, the protein content increased 24 per cent. at the higher temperature. A similar increase occurred in the protein content of the straw.

Soil cultures (in climatic houses)

Series 7.

After reaching the seed-filling stage in the general greenhouse under conditions similar to those of the previous series these cultures were divided into two groups. These were matured in the controlled houses which contained the culture chambers. They were transferred thereto on January 31. The humidity difference was not excessive in these two sections. A test in the closed houses gave vapor pressure deficits of 7 and 10 mm., with much closer values when outside air was admitted for temperature control. No atmometric records were taken. The data from the crops are given in table XIII.

The growth of this series covered the period December 1, 1925, to February 23, 1926. Climatic data were as follows: Temperatures, about 20° and 24° C.; artificial illumination, none; total solar radiation, 6,060 calories;[9] mean hourly maximum solar radiation, 1,820 foot candles; mean

[9] Based on photometric indications that the house value was 0.5 of outdoor illumination.

TABLE XIII

YIELD AND PROTEIN CONTENT OF SERIES 7. GROWTH PERIOD, 85 DAYS. AVERAGE SOLAR ILLUMINATION OF 8-HOUR DAY, 1,240 FOOT CANDLES

TEMPERATURE	NITRATE SUPPLY	YIELD OF SEED	PROTEIN CONTENT OF SEED	YIELD OF PROTEIN	YIELD OF STRAW	PROTEIN CONTENT OF STRAW	YIELD OF PROTEIN
° C.		Gm.	Per cent.	Cgm.	Gm.	Per cent.	Cgm.
16	Liberal	2.13	19.5[a]	42	13.5	6.0[b]	81
24	Liberal	2.04	23.5	48	12.2	5.0	61
16	Scanty	2.27	18.5	42	13.0	5.8	75
24	Scanty	1.79	24.5	44	12.5	4.9	61

[a] Total N × 6.25.

[b] Insoluble and coagulable N × 6.25.

hourly solar radiation, 1,240, based on an 8-hour day; evaporation estimated at not over 40 per cent. higher at the higher temperature than the lower one.

The results show clearly that temperature predominated over the supply of nitrate in determining yield and composition. At the lower temperature the yield of grain increased somewhat, while the protein percentage was considerably decreased. The yield of protein increased a little with the temperature. The yield of straw varied in the same manner as that of the seed, being favored by the lower temperature. In percentage and yield of protein, however, the organs responded quite differently. These functions were enhanced in the straw by the lower temperature. It appears that the production of protein in the straw is favored by the lower temperature, as the data indicate little difference in total transference of this constituent to the seed.

HUMIDITY VARIATION WITH SOIL CULTURES

SERIES 8.

In view of the rôle attributed by agronomists to aridity of climate in affecting the composition of grain, it seemed desirable to compare this factor with temperature. Soil cultures of wheat were brought to seed formation in the greenhouse on optimal planes of water. They were then matured with exposure to uniform illumination and temperature but different atmospheric humidity, in the climatic chambers. The illumination consisted of the maximal sunlight admissible without serious upset of temperature control, supplemented by all the artificial light available. During the 3 to 4 day interims between applications of water to the cultures, the soil moisture varied rather widely in response to the influence of

the atmospheric humidity, as in nature. The data of yield and composition appear in table XIV.

This series grew from November 10, 1925, to February 19, 1926, being transferred to the climatic chambers on January 26. The climatic data follow: Temperature, 13° to 15° C.; relative humidity, about 70 and 40 per cent., equivalent to approximately 3.8 mm. and 7.6 mm. respectively in saturation deficit; evaporation from the standard black atmometer, 82 cc. and 226 cc. respectively; thus, the evaporation in the drier atmosphere was 176 per cent. in excess of the other; maximal artificial illumination, 680 foot candles; total solar radiation, 5,140 calories in the house and 790 in the chamber; mean hourly maximum of solar radiation, 740 foot candles;[10] mean hourly solar radiation (based on 8-hour day), in house 1,130 foot candles, (on 9-hour day) in chambers 510 foot candles.

TABLE XIV

YIELDS AND PROTEIN CONTENT OF SERIES 8. GROWTH PERIOD 102 DAYS. AVERAGE TOTAL ILLUMINATION OF 8-HOUR DAY, 1,140 FOOT CANDLES

RELATIVE HUMIDITY	NITROGEN SUPPLY	YIELD OF DRY GRAIN	PROTEIN CONTENT OF GRAIN	YIELD OF PROTEIN
Per cent.		Gm.	Per cent.	Cgm.
40	Liberal	2.06	18.2	38
70	Liberal	1.79	18.6	33
40	Scanty	1.87	18.6	35
70	Scanty	2.62	17.9	47

The results indicate a favorable effect upon seed yield of the higher plane of atmospheric humidity, with the nitrate supply limited. In this case, also, the protein yield was greatest. The differences in composition were relatively slight, however, and one may conclude that atmospheric humidity exercises much less influence than temperature upon the metabolism of wheat. This is in agreement with the observations of WASSILIEFF (**19**) upon *Lupinus albus*. The test should be repeated with greater illumination.

EFFICIENCY OF ILLUMINATION

For this test wheat and buckwheat were reared on soil in separate semicircular pans of galvanized iron, constructed to fit the rotating tables of the chambers. In this way large numbers of plants were obtained per culture. The first crop grew from November 10 to 24, 1925, after emergence from the soil. The climatic data follow: Temperature, 16°–17° C.; relative

[10] Adjusted for increased illumination before entering chambers.

humidity about 60 per cent.; artificial illumination, 340 and 680 foot candles; total solar radiation, 470 calories; mean hourly maximum of solar radiation, 760 foot candles; mean hourly solar radiation of 8-hour day, 550 foot candles; evaporation, 12 per cent. excess at the lower illumination. The analyses were performed on the undried tissues.

TABLE XV

RELATION OF ILLUMINATION TO DEVELOPMENT. GROWTH PERIOD, 15 DAYS. TEMPERATURE, 16–17° C. ARTIFICIAL ILLUMINATION OF 14-HOUR DAY, 340 AND 680 FOOT CANDLES

PLANT SPECIES	MEAN TOTAL ILLUMINATION, 14-HOUR DAY	DIMENSIONS OF STEM		LEAF PRODUCT	AVERAGE DRY WT. PER PLANT	NITROGEN CONTENT OF DRY MATTER
		LENGTH	DIAMETER			
	Foot candles	mm.	mm.	sq. mm.	mg.	Per cent.
Wheat	650	64	1.4	580	20	5.2
Wheat	990	61	1.5	760	23	5.4
Buckwheat ...	650	182	1.8	290	28	2.2
Buckwheat ...	990	191	2.6	420	19	3.9

The results appear in table XV. Both species of plants responded to the higher plane of illumination by increased leaf area, and the buckwheat attained a correspondingly greater diameter of stem. The yield of wheat increased somewhat without much change in nitrogen content. In the case of buckwheat, we attribute the low yield and high nitrogen content of the tissue produced with higher illumination to increased development of embryonic tissue from the seed reserves, with limited development of photosynthesis.

A second test was extended over a longer period, but in the lower solar light intensity of December 11 to January 4, 1926. Each culture supported 150 plants. The only differences in environmental conditions as compared with the preceding test were in solar radiation. These values were here as follows: Total, 570 calories; mean hourly maximum, 640 foot candles; hourly mean of 8-hour day, 400 foot candles. The appearance of the plants is shown in figure 3, and the yield and composition are given in table XVI.

The yields of dry matter indicate a more serious deficiency of illumination for buckwheat than for wheat. On the other hand, if one assumes the content of sucrose as storage material to be an index of light requirements, the wheat responded more than buckwheat to the increase of illumination. The contents of both total nitrogen and reducing sugars indicate that the lower plane of illumination was here inadequate.

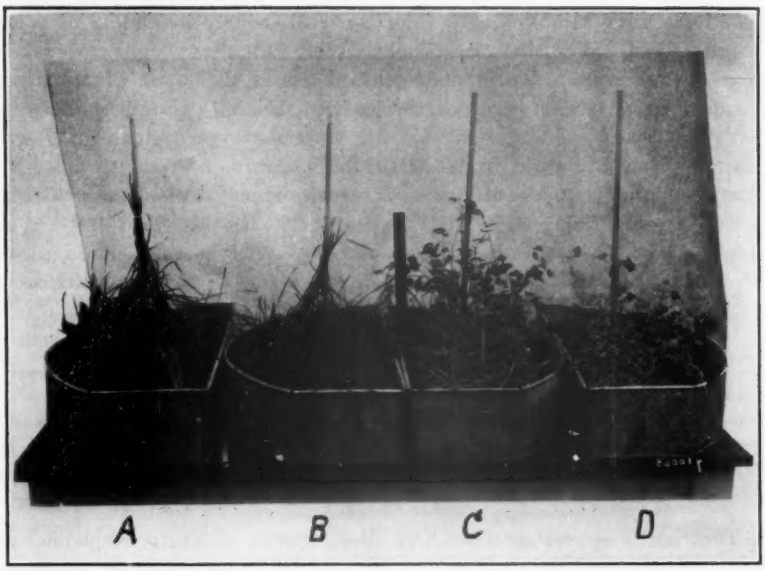

FIG. 3. Relation of illumination to development. Cultures of table XVI tied only for photographing. A. Wheat under higher illumination. B. Wheat under lower illumination. C. Buckwheat under higher illumination. D. Buckwheat under lower illumination.

TABLE XVI

RELATION OF ILLUMINATION TO DEVELOPMENT. GROWTH PERIOD, 25 DAYS. TEMPERATURE 16–17° C. ARTIFICIAL ILLUMINATION OF 14-HOUR DAY, 340 AND 680 FOOT CANDLES

PLANT SPECIES	MEAN TOTAL ILLUMINATION, 14-HOUR DAY	DRY MATTER	TOTAL NITROGEN OF DRY MATTER	REDUCING SUGAR OF DRY MATTER	SUCROSE OF DRY MATTER
	Foot candles	Gm.	Per cent.	Per cent.	Per cent.
Wheat	570	3.48	4.3	0.3	1.3
Wheat	910	4.20	4.1	0.9	2.1
Buckwheat	570	2.74	4.2	0.4	0.7
Buckwheat	910	4.59	4.0	0.9	0.8

Further tests are necessary to establish the minimum requirement of wheat under the conditions of our cultures. The above results indicate a possible deficit of illumination for wheat at about 1,100 foot candles over a 12-hour day. It will be necessary to consider the quality of the light also.

In these tests it was relatively deficient in the shorter wave lengths. We have preliminary evidence that these rays of sunlight or of the light from a carbon arc are important in the assimilation of nitrogen from nitrates.

Temperature effects in young plants

It might well be expected that one could detect in the composition of the young plant indications of such temperature effects as become pronounced at maturity. For investigating this point the tissues should be analyzed from the succulent condition, thus avoiding rearrangements likely to ensue in drying.

To test this matter wheat was grown in water cultures on the full and 0.2N solutions. Forty-five seedlings were reared in each 3.5 liter jar, employing the maximum of artificial radiation available. Growth covered the period March 5 to April 3, 1926, on the 0.2N solution, and 4 days longer on the full one. The effective climatic data were as follows: Temperature 13° to 15° C. and 18° to 20° C.; maximal artificial illumination, 850 foot candles; total solar radiation 2,040 calories and 2,250 calories for the respective sub-series; mean hourly maximum of solar radiation 1,270 and 1,340 foot candles, respectively; mean hourly solar radiation (basis of 10-hour day) 950 foot candles and 925 foot candles respectively; evaporation from standard atmometer 411 cc. at the lower temperature and 402 cc. at the higher, in the first sub-series, 447 and 451 cc. respectively in the longer test. Thus the conditions for evaporation were practically equivalent at the two temperature ranges.

A second test was run from April 19 to May 13 and 14, substituting 0.1N for the 0.2N solution. All factors excepting solar radiation were practically of the same values as in the preceding test. These data are as follows: Total solar radiation 2,400 and 2,450 calories at the respective end dates; mean hourly maximum solar radiation 1,630 foot candles to May 13; mean hourly solar radiation (basis of a 13-hour day) 1,040 foot candles; evaporation to May 13, 248 cc. at the lower temperature and 258 at the higher; thus the increase of evaporation was only 4 per cent. at the higher temperature.

Solar radiation during the measured absorption of nutrients were as follows: Test A, full solution, 324 calories; 0.2N, 333 calories. Test B, full solution, 498 calories; 0.1N, 428 calories. In test B the vacuolar sap and protoplasmic extract were separated by the method of Chibnall (**2**). In all cases the production of dry matter was greater at the higher temperature, as follows: April 3, low temperature 10.2 gms., high temperature 14.3 gms. April 7, low temperature 14.2 gms., high temperature 23.1 gms. May 13, low temperature 13.2 gms., high temperature 13.4 gms. May 14, low

TABLE XVII

COMPOSITION OF SEEDLINGS[a] AND UTILIZATION OF NITRATE AT DIFFERENT TEMPERATURES. GROWTH PERIODS: TEST A, 30 DAYS; TEST B, 25 DAYS. AVERAGE TOTAL ILLUMINATION: TEST A, 1,600 FOOT CANDLES IN 13-HOUR DAY; TEST B, 1,890 FOOT CANDLES IN 13-HOUR DAY

TEST	PLANE OF NITRATE	TEMPERATURE	RED. SUGAR	SUCROSE	PENTOSAN	INSOLUBLE PROTEIN	SOLUBLE PROTEIN	NITROGEN BASES[b]	MONO AMINO ACIDS[c]	NITRATE	ABSORPTION OF NITRATE
		° C.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
A	0.2N	13–15	6.0	2.2	0.8	10.0	5.5	0.9	5.6	2.3	60
A	0.2N	18–20	4.4	0.8	0.8	5.0	3.4	0.9	3.8	3.9	50
A	Full	13–15	2.9	12.1	0.8	10.6	3.1	0.8	4.2	3.9	44
A	Full	18–20	2.5	4.8	0.4	6.3	3.8	0.7	3.0	3.7	49
B (sap)	0.1N	13–15	1.9	4.1	0.6	.....	0.5	0.2	1.5	14.3	74
B (sap)	0.1N	18–20	1.9	2.6	0.5	.....	0.4	0.3	0.8	5.0	70
B (extract)	0.1N	13–15	0.4	0.4	0.9	15.1	1.0	0.5	1.5	.....	.....
B (extract)	0.1N	18–20	0.2	0.5	2.0	8.1	0.8	0.4	1.7	.....	.....
B (sap)	Full	13–15	2.1	4.8	0.5	.....	0.4	0.6	2.5	4.6	−32[d]
B (sap)	Full	18–20	1.7	3.9	0.6	.....	0.4	0.6	1.9	4.3	−33
B (extract)	Full	13–15	0.6	2.4	0.8	10.2	1.4	0.3	1.3	.....	.....
B (extract)	Full	18–20	0.6	1.5	0.8	6.4	0.8	0.3	0.9	.....	.....

[a] Percentages based on dry matter.

[b] N of protein-free phosphotungstic acid precipitate × 5.

[c] N of filtrate from basic nitrogen × 5.

[d] Negative balance. 32–33 per cent. increase in the solution.

temperature 13.6 gms., high temperature 15.7 gms. The results of both series appear in table XVII.

These data show irregularities in trend but, on the whole, seem to justify the following generalizations: Sucrose and proteins were synthesized to a greater extent at the lower range of temperature. This was true to a lesser degree of reducing sugars and amino acids. A similar apparent dependence of nitrate assimilation upon the occurrence of sugars was noted in earlier work with the sugar beet (**18**). The absorption of nitrate at the lower planes of supply was favored by the lower temperature. At the higher plane of supply this relation seems to be reversed. The negative absorption of nitrate at the full plane of supply in test B is correlated with low solar illumination. On the day of sampling and the preceding day the mean hourly solar radiation in the chambers was only 500 and 420 foot candles respectively (1,350 and 1,270 foot candles total illumination). Soluble pentosans and basic forms of nitrogen formed relatively constant proportions of the tissue. As might be expected from the artificial mode of its determination, soluble protein shows no regular response to temperature. Indeed, the significance of attempts to distinguish between soluble and insoluble protein in such foliage extracts seems highly questionable. The protein content of the dry roots (insoluble and coagulable nitrogen $\times 6.25$) was determined for the full nutrient solutions in both series of cultures. The results were as follows:

April 7, low temperature 13.9 per cent., high temperature 13.3 per cent.
May 14, low temperature 12.1 per cent., high temperature 13.2 per cent.
These results are rather uniform.

Discussion

The preceding details give some metabolic responses of the wheat plant to rather completely circumscribed environmental conditions. Considering the limitations placed upon other factors, the degree of regulation of temperature was satisfactory. It appears that rather wide fluctuations of atmospheric humidity have little influence upon composition during maturation of the plant.

Illumination is in need of further investigation as regards both quality and intensity. Nevertheless, the tests here reported in which illumination was probably a limiting factor should possess some value inherent in a defined environment. The large proportion of sunlight employed entailed wide variations in total light intensity and distribution of wave lengths in the course of some tests, as well as in different culture series. In subsequent work it is purposed to employ light only from artificial sources. The results of these several series of cultures are summarized in table XVIII,

TABLE XVIII

SUMMARY OF CHIEF CONDITIONS AND RESULTS IN TEMPERATURE EFFECTS ON FULL NUTRIENTS

Series	Plant part	Temperature range	Average plane of total illumination 12-hour day	Duration	Relative yield and composition at lower temperature as compared with higher one				
					Dry matter yield	Carbohydrates		Proteins	
						Percentage	Yield	Percentage	Yield
		° C.	Foot candles	Days	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1	Straw	10	390	41	155	170[a]	265	100[e]	150
2	Straw	10	1010	41	90	68[a]	61	135[f]	117
2	Roots	10	1010	41	144	140[b]	200	87[f]	123
3	Straw	10	530	62	130	54[c]	70	178[f]	233
3	Roots	10	530	62	119	90[c]	107	67[f]	78
4	Straw	5	1150	80	324	.....	.....	95[f,g]	309
5	Seeds	6	1560	99	246	.....	.....	104[e]	256
5	Straw	6	1560	99	184	.....	.....	114[h]	210
6	Seeds	6	2510	82	152	.....	.....	80[e]	122
6	Straw	6	2510	82	112	.....	.....	77[h]	86
7	Seeds	8	830	85	104	.....	.....	83[e]	88
7	Straw	8	830	85	111	.....	.....	120[f,g]	133
A	Total extract	5	1730	30	61	205[d]	126	136[f,g]	84
B	Sap	5	2050	25	104	123[d]	107	100[g]	87
B	Extract	5	2050	25	104	143[d]	124	161[f,g]	140

[a] Hemicellulose.
[b] Total sugar and hemicellulose.
[c] Reducing sugar and hemicellulose.
[d] Total sugars.
[e] Crude protein.
[f] Insoluble protein.
[g] Soluble protein.
[h] Nitrogen compounds precipitable by phosphotungstic acid.

with illumination values converted to a uniform time basis. Without reference to the character of light, the following culture series were either certainly or possibly deficient in intensity of illumination, namely, 1, 3, and 2, 4, 7 respectively, with maxima of 530 and 1,150 foot candles over a 12-hour day. In most of these series the production of straw was decidedly favored by the lower temperature. The only crop in this group which produced seed (series 7) was reared outside the chambers. Its grain contained a considerably greater percentage of proteins at the higher temperature and a somewhat higher yield of protein. The other series (series 4) reared to maturity seems to have been disturbed in seed production by unfavorable temperatures for pollination, rather than by deficient illumination. At these lower planes of illumination the percentage of protein in the straw was generally favored by the lower temperature at the expense of available carbohydrates. Where carbohydrate determinations were omitted, it seems reasonable to assume that their percentages varied inversely as the larger differences of protein.

The remaining culture series (5, 6, A and B, with total light intensities of 1,560 to 2,510 foot candles over a 12-hour day) may be assumed to have received fairly adequate illumination, until proved otherwise. Plants harvested at one-third to one-fourth their full growth periods (series A and B) produced somewhat more tissue at the higher temperature. However, the increased percentages of sucrose and protein at the lower temperature gave evidence of increased storage capacity therefor. It seems probable that this function is determined by the plane of sugars, the latter then determining the plane of protein synthesis. This interpretation has support in the generally increased yield of protein, as well as of carbohydrates at the lower temperatures. The yield of both grain and straw from mature cultures (series 5 and 6) were greater at the lower temperature, the effect being marked with the grain. Increased proportions of carbohydrates in the tissues was also characteristic of the lower temperature.

The results here discussed apply to fully nourished plants. With a reduction in the supply of nitrate this nutrient generally became a limiting factor, especially at the higher temperature. In these several responses one generalization appears rather distinctly, namely: The accumulation of carbohydrates and proteins, as to quantity, is dependent upon growth at lower temperatures.

With increasing emphasis conditions have pointed toward the balance between synthesis and respiratory decomposition as the determinant of such compositional effects. The respiratory response is being measured. Meanwhile, LUNDEGARDH (**13**) has presented convincing evidence that this balance becomes critical with increase of temperature. It is well known that the

[h] Nitrogen compounds precipitable by phosphotungstic acid.

respiratory activity of plants increases with rise of temperature. The rate of increase is two-fold or greater at 15° to 25° C., while the increase of photosynthesis, as thus far determined, is appreciably smaller. Increase of temperature should thus result in decrease of the carbohydrate: protein ratio, as observed in the present work.[11] McGinnis and Taylor (**15**) take exception to this mode of interpretation, but the validity of their supposedly contradictory evidence is open to serious question. Working with detached seeds, they failed to take into account the determining effects of temperature upon the ratio of carbohydrate to protein in the reserves of leaf and stem, which supply the developing grain. Mangels (**14**) concludes that the chief environmental factor influencing the percentage of protein in wheat is the temperature over several weeks preceding the filling stage. He found higher temperatures correlated with increased percentages of protein in the grain, although the yield of protein was less than at lower temperatures. Our experimental results agree with his statistical findings. The evidence seems to be strongly in favor of the conception that temperature becomes a dominant factor in the regulation of metabolism by controlling the equilibrium between certain anabolic and catabolic reactions. This conception does not preclude recognition of a possible rôle of aridity also in the production of high-protein wheat in cropping practice.

Summary

1. Improvements in apparatus for the control of environmental factors in plant growth are described, together with methods of estimating the total illumination effective from sun and electric lamps.

2. Details are given of the conduct and results with nine series of wheat cultures in water, sand and soil. Each series was subjected to two planes of temperature, but with illumination and atmospheric humidity uniform.

3. Evidence is presented that atmospheric humidity exercises little influence upon the protein content of wheat during the period of seed development.

4. With illumination probably deficient in intensity, lower temperatures favored the production of dry matter, as if conserving limited supplies of photosynthetic products. Under these conditions the lower temperature generally favored both the percentage and yield of protein in the tissues.

5. It is shown that the failure of seed formation in plants thus closely confined is chiefly due to unfavorable conditions for pollination.

6. With illumination probably adequate in intensity, young wheat plants contained greater percentages of sucrose and protein when grown at lower

[11] Since this was written Bushnell (Techn. Bull. 34, Minnesota Agricultural Experiment Station) has presented evidence that increased respiration at higher temperatures results in decreased tuber formation of the potato.

temperatures. Mature plants contained increased percentages of carbohydrates and gave markedly increased yields, especially of grain, at the lower temperatures.

7. The higher sugar content at lower temperatures appears to favor protein synthesis, but at higher temperatures deficiency of nitrate supply seems to operate as a limiting factor.

8. Although the amount of protein synthesized is less at the higher temperatures its percentage in the tissue is increased, apparently because of greater respiratory loss of carbohydrates than at the lower temperatures.

9. In explanation of these metabolic effects the conception is advanced that there is a critical balance between the temperature responses of photosynthesis and respiration.

10. The need of measurement of respiration and photosynthesis in continuance of this work is recognized. Furthermore, the light factor should be made reproducible as to both quality and intensity.

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SULPHUR METABOLISM OF YEAST[1]

H. SUGATA AND FRED C. KOCH

Introduction

Two general conclusions are warranted as to the sulphur metabolism and nutrition in animals. The first is that although most of the sulphur taken into the system is in the reduced form, that is, mainly as cystine or possibly also as cysteine, nevertheless the main end product of normal sulphur metabolism is the completely oxidized sulphate form. This is well established by numerous investigations. The other important conclusion is that cystine is a very important amino acid in growth and maintenance.

Recently the importance of sulphur in nutrition of plants has also been so well shown that the use of sulphates in fertilizers is emphasized. ARMSTRONG (1) also studied the effect of various sulphur compounds in the development of *Aspergillus niger, Penicillium glaucum,* and *Botrytis cinerea.* He concluded that the best form of food sulphur is sulphate, and that even here the end product of sulphur metabolism is the sulphate form.

Recent studies (7) in this laboratory indicate that cystine also may be of some importance in yeast growth and that studies on yeast, when properly controlled, may throw more light on certain phases of metabolism. The object of this investigation was to determine more definitely the forms of sulphur available for yeast growth, and to investigate further the changes cystine undergoes in yeast growth and metabolism.

In all the studies here reported the concentration of the yeast growth stimulant was carefully controlled just as in the work by MILLER (5) and SWOBODA (7). In the first part of the work various forms and amounts of sulphur, as found in biological material, were added to an artificial medium containing a known, but very small amount of sulphur, and the rate of yeast growth determined by the weighing method. In the second part of the study the yeast was separated and analyzed for sulphur, and the filtrate analyzed for total sulphur and for "inorganic sulphate."

Experimental

1. MEDIA AND SULPHUR COMPOUNDS.—The following stock media were used from time to time with or without other additions as indicated under the observations made. The materials used in these media and the various

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sulphur compounds added were for the most part prepared or purified in these laboratories.

The saccharose first employed was the commercial rock candy, but we soon found this to contain sufficient amounts of sulphur to influence the results. It was then purified by solution in water and precipitation by redistilled absolute alcohol. By this procedure the sulphur content was reduced from the equivalent of 0.7 mg. to 0.1 mg. barium sulphate per gram of saccharose.

The asparagine was of the C.P. grade but was again recrystallized from water. The inorganic salts used were the "analyzed chemically pure" grade. The cystine was obtained from horn in the usual way and then reprecipitated five times from an aqueous solution of the hydrochloride by ammonium hydroxide. Cysteinic acid was prepared from cystine by FRIEDMANN'S method (**2**). It was recrystallized from hot water and washed with alcohol. Taurocholic acid was prepared from ox bile by HAMMARSTEN'S method (**3**), and taurine therefrom by the HAMMARSTEN (**4**) and TAUBER methods (**8**). The purity of these substances as measured by the sulphur content is indicated in table I.

TABLE I

SULPHUR CONTENT OF MATERIALS USED

SUBSTANCE	$BaSO_4$ OBTAINED PER GRAM	PER CENT. OF SULPHUR	THEORETICAL PER CENT. OF SULPHUR
	mg.		
Purified saccharose	0.1	.....	.....
Asparagine	0.2	.....	.....
Vitamine solution 5 per cent.	0.085	.....	.....
KH_2PO_4	1.4		
Cystine	.....	26.59	26.69
Cysteine	.....	26.59	26.47
Taurocholic acid	.....	5.33*	6.22
Taurine	.....	25.0	25.63
Cysteinic acid	.....	18.88	18.87
Dried yeast	.....	0.35	.....

* The low value is probably due to an admixture of moisture and metallic impurities. An ash estimation confirmed the latter interpretation.

The vitamine solution was prepared by drying Fleischmann's best grade of stock yeast in a current of air at room temperature. This material was next thoroughly ground in a mill and extracted six times with hot absolute

ether. The ether extract was discharged and the yeast powder next extracted six times with boiling 75 per cent. alcohol. After cooling, the combined alcoholic extracts were filtered and then concentrated to a paste under diminished pressure. The material was next redissolved in hot 90 per cent. alcohol and again cooled in the refrigerator and filtered. Thus 500 cc. of stock solution were obtained from five pounds of fresh yeast. The vitamine solution as added to the media in most cases was a 5 per cent. solution of this alcoholic stock solution. The media used are shown in table II.

TABLE II

COMPOSITION OF MEDIA EMPLOYED

SUBSTANCE	MEDIUM NUMBER						
	1	2	3	4	5	6	7
	grams	grams	grams	grams	grams	grams	grams
Saccharose	20.0	20.0	20.0	20.0	20.0	100.0	100.0
Asparagine	1.5	1.5	1.5	.....	1.5	7.5	7.5
$NH_4H_2PO_4$	.....	3.0	.....	.....	.....	.....	.....
KH_2PO_4	2.0	2.0	2.0	2.0	2.0	10.0	10.0
$MgSO_4$	.....	.....	.....	.....	.....	.....	1.20
$MgCl_2$	.....	0.25	0.25	0.25	.....	1.01	.....
$CaCl_2$	.....	0.25	0.25	0.25	0.25	1.25	1.25
Diluted to	1000	1000	1000	1000	1000	500	500

2. GROWTH METHOD.—The methods of WILLIAMS (**9**), MILLER (**5**), and SWOBODA (**7**) were used with slight modifications. A 125 cc. volume of the medium in a 500 cc. Erlenmeyer flask was sterilized in an autoclave at ten pounds for ten minutes and after cooling 1 cc. of the usual yeast suspension was added. After growth at 30° C. for 20 hours a few drops of formaldehyde were added so as to stop the growth of yeast. The yeast cells were at once filtered off into the weighed Gooch crucible, dried in the oven for 30 minutes at 103° C. and weighed after cooling.

3. ANALYTICAL METHOD FOR SULPHUR.—The method of alternate oxidation by hydrogen peroxide and by nitric acid as devised by STOCKHOLM and KOCH (**6**) was employed. It gave excellent checks and theoretical values for the well purified sulphur compounds and is very satisfactory for small amounts of sulphur.

Experimental observations

A. THE IMPORTANCE OF CALCIUM, MAGNESIUM AND SULPHATE IN YEAST GROWTH.—To 100 cc. of medium no. 1 varying amounts of M/10 $CaCl_2$, M/10 $MgCl_2$, M/10 $MgSO_4$, M/10 H_2SO_4 and vitamine solution were added

in various combinations and then diluted to 125 cc. Yeast was then grown in these special media, filtered off, dried and weighed. The results given in table III are with the unpurified saccharose and 5 cc. of the 5 per cent. vitamine solution per 100 cc. medium no. 1. In table IV we have a similar procedure, only here we have one-fifth of the amount of vitamine solution. In table V we have similar observations on medium no. 1 with the purified saccharose. Similar results were obtained repeatedly in connection with many other growth tests.

TABLE III

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 5 CC. VITAMINE IN MEDIUM NO. 1 WITH VARYING SALTS

NUMBER	$MgCl_2$ M/10	$CaCl_2$ M/10	$MgSO_4$ M/10	$(NH_4)_2SO_4$ M/10	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2	...	...	...	17.7
2	...	2	2	...	...	49.1
3	...	...	2	...	1	44.2
4	...	...	...	2	1	1.6
5	2	2	...	...	1	48.5
6	...	2	2	2	...	51.0
7	2	...	2	...	1	47.4
8	2	2	...	2	...	42.1
9	2	2	2	...	...	52.2
10	...	2	2	...	1	48.4

TABLE IV

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 1 CC. VITAMINE IN MEDIUM NO. 1 WITH VARYING SALTS

NUMBER	$CaCl_2$ 1%	$MgCl_2$ 1.5%	$MgSO_4$ 1.5%	$(NH_4)_2SO_4$ 2%	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2	...	...	...	9.6
2	2	...	2	...	...	12.1
3	2	...	1	1	...	12.8
4	2	...	2	1	...	14.4
5	2	...	2	...	1	12.3
6	2	...	4	...	...	11.8
7	2	...	...	4	...	1.2
8	2	...	8	...	...	11.8
9	2	...	...	8	...	1.4
10	2	...	4	4	...	14.6

TABLE V

GROWTH OF YEAST IN PURIFIED SACCHAROSE, WITH VITAMINE IN MEDIUM NO. 1 PLUS SALTS

NUMBER	$CaCl_2$ M/10	$MgCl_2$ M/10	$MgSO_4$ M/10	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	...	...	...	7.0
2	2	2	...	...	7.6
3	2	...	1	...	8.6
4	2	...	2	...	9.9
5	2	2	2	...	11.2
6	2	...	4	...	11.4
7	2	...	8	...	9.7
8	2	2	...	1	9.7
9	2	2	...	2	10.2

B. THE SUBSTITUTION OF REDUCED SULPHUR FOR SULPHATE SULPHUR IN THE MEDIUM.—In the first part of these studies the cystine was dissolved in a KH_2PO_4 solution and this was then added to a modified medium no. 3 together with a standard KH_2PO_4 solution so that the total amount of KH_2PO_4 always remained 2.0 grams per liter. Many series of growth tests were made and in all cases the results were essentially as indicated in table VI. However, if asparagine is not present in the medium the results in

TABLE VI

GROWTH OF YEAST IN MEDIUM NO. 3, PLUS VITAMINE AND CYSTINE AS INDICATED WITH AND WITHOUT ASPARAGINE

NUMBER	VITAMINE SOL. 5 PER CENT.	CYSTINE	WEIGHT OF DRIED YEAST WITH ASPARAGINE	WEIGHT OF YEAST WITHOUT ASPARAGINE IN THE MEDIUM
	cc.	mg.	mg.	mg.
1	...	...	2.9	0.5
2	1	...	8.9	2.5
3	1	1	10.0	.....
4	1	2	10.5	2.2
5	1	3	12.4	.....
6	1	4	14.3	2.5
7	...	1	3.0	.....
8	...	2	3.6	1.3
9	...	3	4.2	.....
10	...	4	5.1	1.3

the last column of table VI are obtained. By adding various mixtures of the salts and cystine to medium no. 6, together with vitamine in each case,

the values in table VII were obtained. Practically the same observations are given in table VIII, where medium no. 6 also was used as the stock medium with 1 cc. vitamine solution in each test.

TABLE VII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS VITAMINE WITH SALTS AND CYSTINE ADDED, AS INDICATED

NUMBER	$MgCl_2$ M/10	$MgSO_4$ M/10	NH_4Cl M/10	CYSTEINE	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.	mg.
1	2	...	...	...	9.2
2	2	...	...	1	10.7
3	2	...	...	3	11.4
4	...	2	...	...	14.4
5	...	2	...	1	14.9
6	...	2	...	3	14.5
7	2	...	2	...	11.2
8	2	...	2	1	11.7
9	2	...	2	3	12.0
10	2	...	20	1	10.4
11	2	...	20	3	11.2

Cysteine, used in amounts ranging from two to twenty milligrams, gave results very similar to cystine.

TABLE VIII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, AND WITH SALTS AND CYSTINE ADDED

NUMBER	$MgCl_2$ M/10	$MgSO_4$ M/10	NH_4Cl M/10	CYSTINE	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	...	...	...	8.6
2	2	...	...	1	10.2
3	2	...	...	3	13.9
4	2	...	...	10(?)	10.4
5	...	2	...	...	14.3
6	...	2	...	1	16.5
7	...	2	...	3	13.3
8	...	2	...	10(?)	12.7
9	2	...	20	...	8.2
10	2	...	20	1	10.9
11	2	...	20	3	12.8
12	2	...	20	10(?)	12.4

Hydrogen sulphide seems to be able to supply sulphur for yeast growth to a limited extent. The results, using medium no. 6 as the stock medium, are given in table IX. In each case 1 cc. of the vitamine solution was added.

TABLE IX

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, WITH SALTS, AND H_2S ADDED AS INDICATED

NUMBER	$MgSO_4$ M/10	$MgCl_2$ M/10	H_2S 1 cc. = 0.00216 mg.	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.
1	2	...	...	13.1
2	2	...	1	10.8
3	2	...	2	10.9
4	2	...	4	10.2
5	...	2	...	8.4
6	...	2	1	10.7
7	...	2	2	11.0
8	...	2	4	9.9
9	...	2	6	10.2
10	...	2	10	7.3
11	...	2	20	6.9

C. THE VALUE OF SULPHONATE SULPHUR.—In tables X and XI are given the results obtained with taurocholic acid, cysteinic acid and taurine. In table X are given the results, using medium no. 3 and no. 4 as stock media. In table XI the stock medium was no. 5.

TABLE X

GROWTH OF YEAST WITH TAUROCHOLIC ACID AS SULPHUR SOURCE

NUMBER	5 PER CENT. VITAMINE SOLUTION	TAUROCHOLIC ACID ADDED	MEDIUM NO. 4 WEIGHT OF DRIED YEAST	MEDIUM NO. 3 WEIGHT OF DRIED YEAST
	cc.	mg.	mg.	mg.
1	1	0	8.5	12.3
2	1	1	8.6	11.5
3	1	4	6.3	10.2
4	1	10	5.0	7.1
5	0	0	2.6	3.0
6	0	1	2.5	1.8
7	0	4	1.6	1.5
8	0	10	1.1	1.3

TABLE XI

GROWTH OF YEAST IN MEDIUM NO. 5, CYSTEINIC ACID AND TAURINE TESTS

NUMBER	5 PER CENT. VITAMINE SOLUTION	$MgCl_2$ M/10	$MgSO_4$ M/10	CYSTEINIC ACID	TAURINE	AVERAGE WEIGHT OF YEAST
	cc.	cc.	cc.	mg.	mg.	mg.
1	1	2	...	...	...	9.0
2	1	2	...	2	...	10.6
3	1	2	...	6	...	10.0
4	1	2	...	10	...	9.9
5	1	2	...	20	...	9.7
6	1	2	...	...	2	9.3
7	1	2	...	...	6	9.5
8	1	2	...	...	10	9.7
9	1	2	...	...	20	10.2
10	1	...	2	...	...	14.9
11	1	...	2	2	...	13.4
12	1	...	2	6	...	11.0
13	1	...	2	10	...	10.4
14	1	...	2	20	...	9.9
15	1	...	2	...	2	14.6
16	1	...	2	...	6	14.6
17	1	...	2	...	10	13.9
18	1	...	2	...	20	14.3

D. UTILIZATION OF INORGANIC SULPHATE SULPHUR.—Yeast was grown in 500 cc. of medium no. 7 with the addition of 5 cc. vitamine solution. After the usual growth period the yeast was separated by centrifuging and washed twice by centrifuging and decanting. After drying and weighing, the total sulphur was estimated by the method referred to previously. The filtrate was carefully recovered and the "total sulphate" estimated by the usual method. The original medium contained 0.0244 grams sulphur as sulphate; the filtrate from the yeast contained 0.0225 gram or a difference of 0.0019

TABLE XII

UTILIZATION OF CYSTINE SULPHUR BY YEAST, MEDIUM NO. 6

SUBSTANCE	TOTAL SULPHATE	TOTAL SULPHUR	CYSTINE SULPHUR	SUM
	gm. S	gm. S	gm. S	gm. S
Original medium	0.00137	.....	0.0127	0.01407
Yeast filtered off (0.6752 gram)	.....	0.00462	.....	0.00462
Filtrate from yeast	0.00541	.....	0.00322	0.00863

gram sulphur. This amount apparently was removed from the medium during growth. The yeast filtered off contained 0.0016 gram sulphur.

E. UTILIZATION OF CYSTINE SULPHUR.—In this case 0.05 gram cystine was added to 500 cc. of medium no. 6 and the sulphate, cystine and total sulphur determined in the original medium and in the filtrate after growth. The cystine was determined by the phosphotungstic acid precipitation method as employed in the VAN SLYKE procedure for protein analysis. The results are given in table XII.

Discussion of results

A comparison of tables III and IV first of all shows the stimulating action of increased amounts of vitamine under otherwise constant conditions. Both of the tables very clearly show the value of both magnesium and sulphate in yeast growth; and it is particularly noteworthy to observe that in the presence of a liberal supply of the growth-stimulating vitamine, the effect of the same addition of magnesium and sulphate is eight to ten times that observed with a low vitamine content. The results also show that the optimum concentration of magnesium is very soon attained even where growth is extensive as shown in table III. An addition of magnesium beyond that amount neither improves nor injures the medium, no matter whether the vitamine concentration is low or high. The same general conclusions hold for sulphate. Comparison of no. 4 and no. 3 in table III very strikingly shows how much more important magnesium is in this medium than the ammonium. Calcium does not appear to be nearly so important as magnesium.

The observations on the effect of sulphate clearly show that growing yeast is able to utilize sulphate and presumably convert it into protein (probably cystine) sulphur. The quantitative studies on the loss of sulphate from a sulphate medium confirm the same general conclusions as to the utilization of sulphate in yeast growth.

The studies with cystine again confirm the slight stimulating effect thereof when in the lower concentrations, but that one can soon observe an inhibiting effect when the concentration is increased beyond say five to ten milligrams per 125 cc. of medium. This inhibiting effect is observed much more readily when sulphate also is present in the medium. In a sulphate-free medium an otherwise minimal inhibiting concentration of cystine is still stimulating. So also the stimulating effect of cystine is much more marked in a medium containing asparagine than in one free therefrom. In no case, however, could cystine sulphur be satisfactorily substituted for sulphate sulphur. An amount of cystine, equivalent in sulphur content to a good concentration of sulphate as magnesium sulphate or ammonium

sulphate, is toxic in any case. Thus ten milligrams of cystine contain 3.6 milligrams of sulphur whereas 6.4 milligrams of sulphur as sulphate is a very favorable concentration. The observations on cysteine lead to the same general conclusions.

Hydrogen sulphide can also be drawn upon for yeast growth in the absence of sulphate, but one soon reaches a toxic concentration which is far below the sulphur content of a desirable sulphate concentration. Whether the sulphide itself is exceedingly toxic and only that part of it which has become oxidized in the medium is utilized by the yeast, or whether a trace of the sulphide is the real intermediate form between sulphate and yeast tissue sulphur is not determined. Here again an extremely low concentration of hydrogen sulphide (0.00216 milligram H_2S per 125 cc.) is toxic to yeast growth when sulphate is added to the medium, but when the sulphate has not been added it requires ten times the concentration of hydrogen sulphide before a toxic effect is observed. However, even then the stimulating effect is very slight indeed. The action of hydrogen sulphide is very similar to that of cystine, but it is very much more toxic. Possibly cystine itself is harmless, but hydrogen sulphide liberated from it in traces may be the real reason for the apparent toxicity of cystine.

Apparently the yeast is able to protect itself against cystine to some extent by oxidizing the sulphur in cystine to the sulphate form. This is shown in table XII. This observation is very interesting because the yeast metabolism of excess cystine appears to be very similar to that in man where the main excretory form is sulphate. The results also indicate that of the cystine sulphur which was changed, approximately equal amounts were found in the yeast as yeast protein and in the filtrate as sulphate respectively.

The results with taurocholic acid, cysteinic acid and taurine indicate that taurocholic acid is toxic in any case no matter whether other more available forms of sulphur are present or not. Cysteinic acid aids only slightly in an otherwise sulphur-free medium; and in the other media, containing more available forms of sulphur, it is toxic. Cysteinic acid thus stands between cystine and taurocholic acid. Taurine is without appreciable action in the concentrations employed. These results suggest that the cholic acid part in taurocholic acid introduces the toxic effect, but also that the sulphonate form of sulphur is the least available for yeast growth.

Summary and conclusions

1. Magnesium and sulphate appear equally important in yeast growth.

2. Of the forms of sulphur studied, the inorganic sulphate form is the most available.

3. Sulphate sulphur is a true nutrient in that it is actually converted into yeast protein and probably at least in part into cystine.

4. Cystine, cysteine, and hydrogen sulphide stimulate yeast growth in a sulphate-free medium up to certain, but low concentrations. Above those concentrations they retard growth. Cystine also stimulates slightly in a sulphate-containing medium, but hydrogen sulphide retards growth in such a medium even in extremely low concentrations.

5. When yeast grows in a sulphate-free medium containing cystine as the source of sulphur it converts part of the cystine into new yeast protoplasm and about an equal amount into sulphate left in the medium.

6. Taurocholic acid in very low concentrations retards yeast growth. Cysteinic acid acts intermediate between taurocholic acid and cystine.

7. Taurine is without action on yeast growth in the concentrations here employed.

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STUDIES ON THE OXIDATION OF CERTAIN FATTY ACIDS

J. B. RHINE

Introduction

In both plant and animal bodies, under certain conditions, sugars are changed to fats; and in the plant, if not in the animal, these fats are converted back to sugars for use when a certain other set of conditions is provided. These are well known facts of physiology. It is pretty well established that the point of departure is the fatty acid; that is, the fatty acid is formed before the neutral fat, and the neutral fat is hydrolized to fatty acid and glycerol before being changed to sugar. Thus we may consider the changes as one between fatty acid and sugar for our present purposes. This reversible transformation is one that is very common and widespread in the plant kingdom, not to mention the animal world. Great quantities of food are put through this process, especially in the ripening and germination of fatty seeds. In these cases the carbon is conducted as a soluble carbohydrate and then stored in the lighter, more compact, and insoluble form of fat. Before being transportable again and ready for use in other parts of the plant it must be made soluble again; the common transport material of the fatty seedling is sugar.

But these very common food transformations are not understood. We know that at one time we have sugar and at another, fat, or vice versa; but we do not understand how this remarkable change can be so generally brought about especially at the normal air temperatures. This general biological interchangeability of fats and sugars has no chemical parallel, nor have chemists been able to point out the manner in which such a transformation can be made, although it is a question of much simpler compounds, structurally, than some that have been successfully dealt with in the laboratory. Of course, we know that the formation of the fatty acid requires a reduction of the carbon atom, and the change back to sugar is an oxidation process; and we know further that in so changing, it has attached an atom of oxygen per carbon atom. Anything further on the intermediate steps is purely hypothetical and not supported by evidence.

The fact that enzymes have been isolated which are found to aid in the reactions of many of the known plant processes, naturally suggests that there may be one or more enzymes responsible for the changes here under consideration. It must be remembered, however, that there is probably much to protoplasmic activity that is not to be detached, so to speak, not

performed by separate agents like enzymes. No doubt some of the more fundamental processes are inherent in the very nature and structure and activity of the protoplasm itself. The sugar-fat-sugar processes may be of this order. At any rate, this must be borne in mind in the interpretation of negative results in searching for enzymes for these processes.

The most logical attack on the general problem seemed to be an attempt to discover if an enzyme could be isolated which would bring about a part or all of these changes *in vitro*. It seemed best, too, to work with the fatty acid-to-sugar half of the process for the following reasons: That the oxidation phase seemed likely to occur more easily *in vitro*; that the products would probably be more easily tested for; that starting with long chain fatty acids involved less danger of contamination with organisms than starting with sugars; etc. Obviously, even this single phase of finding an enzyme which changes fatty acids to sugars represents an extensive undertaking and probably could never be settled in the negative, so many are the possibilities to be exhausted. The enzyme, if any, can only be sought for with such general methods as are available, adding such special modifications as may occur, and continuing the search until it no longer seems profitable, or until it is found.

Experimental

In 1923 while at Hull Botanical Laboratory of the University of Chicago an attempt was made to find an enzyme responsible for this reaction. Brief mention of this attempt is made by way of introduction to what is to follow. Mixed fatty acids from linseed oil were used chiefly, although not entirely. The extractions for the enzymes were made for the most part upon germinating fatty seeds, and in general the methods of extraction were those used to prepare lipase, although other methods were tried, even to using the filtered and dialyzed expressed juice of the seedlings. Small amounts of fatty acids were put in small flasks, with the water solution of the extract slightly acidified with HCl (using 1 part of fatty acid to about 10 parts of extract solution), shaken up frequently, and kept in a 40° C. oven. In some cases thymol, in others chloroform, and in others nothing was added to prevent the activity of organisms. No signs of such activity were ever present however. A check on the changes going on in the extract itself was made in each case, and a check on the fatty acids also was made each time, using acidified distilled water in place of the extract. They were allowed to stand for various periods of time, up to three weeks. The preliminary test for results used was reduction of Fehling's solution by the water layer in the flask.

No reduction of Fehling's solution was obtained, however, which was not also obtained from the check flask that did not contain the extract.

Only a slight amount of reducing substance was present at all, but the same degree of reduction was produced in the flask containing only the fatty acids and the acidified distilled water. The amount of substance was too small to use in further testing for its nature. But, although the experiments failed in their main objective, they made evident the fact that these fatty acids were undergoing a decomposition of some kind, yielding a water soluble, reducing substance. It could not, of course, be called a sugar on such evidence, but the facts were sufficient to warrant further investigation of the nature of this substance, and the process by which it was produced. The enzyme work was merely laid aside then, not settled.

In 1924, at the Boyce Thompson Institute for Plant Research a study was undertaken of the decomposition of fatty acids which was apparent from the enzyme experiments mentioned. The object was still to throw some light on the processes of the plant by which fatty acids are changed to sugar; the immediate aim was to study the products yielded in this mild oxidation, considering that they might lead to some understanding of the break down of the fatty acid in the organism. The general method was to furnish higher temperatures, increased acidity, and abundance of oxygen to the mixture of fatty acids and water, the water being used to remove the soluble products as they were formed. The method is analogous to the general method for performing, *in vitro*, hydrolyses that normally occur in the organism, substituting for the enzyme, heat and acidity.

To provide these conditions, 100 ml. of the fatty acids and 200 ml. of distilled water (in some cases made up to N/50 sulphuric acid, later in most cases not acidified) were put in an 800-ml. Kjeldahl flask fitted with a reflux condenser. Tube connections leading from a compressed air tank ran to the bottom of the flask so that the air bubbled up continually and kept the two liquids well mixed. The flask was kept at a roughly constant temperature, varying at most 3° C., by means of a sand-bath on an electric hotplate. All runs were made between 75° and 95° C. The current of air, in addition to emulsifying the oil and water, furnished a continually fresh supply of oxygen. In some cases a rapid stirrer was used, with only a slow current of air. This, however, furnished no advantage over the other method of mixing (rapid bubbling of air) and was discontinued. After a few attempts the sulphuric acid also was left out, since it seemed to make no great difference; the water solution soon became acid without it. The strong current of air had the disadvantage of carrying off some of the more volatile products, even through the long reflux condenser. But it was found that most of the volatile products could be caught by connecting with the upper end of the reflux condenser and leading the out-going air through a flask of water, rendered slightly alkaline.

The fatty acids used were prepared from purest raw oils obtainable. They were carefully saponified with alcoholic potash and the fatty acids freed by addition of dilute sulfuric acid to the hot diluted soap. The free acids were repeatedly washed until the washings showed no sign of the mineral acid. The fatty acids were tested for acrolein and for unsaponified fat (Geitel test). These impurities were never found in any of the fatty acids used. The palmitic and stearic acids were obtained on the market, the best available. They were not resaponified because by the time they were used it was clear that the neutral fat was not objectionable in small quantities in this investigation. This is made evident later.

After the treatment of the fatty acids for the desired period, the water layer was separated from the fatty layer by filtering. For general testing, filtering through ordinary filter paper was sufficient; for certain cases, however, it was necessary to use charcoal. Other filters such as asbestos, aluminum crucibles, etc., were tried. It was difficult to completely remove all the finely divided and suspended oil droplets from the solution without the use of charcoal.

The first general test on the products of this treatment of the fatty acids was made with Fehling's solution. As a preliminary test equal parts of the mixed Fehling's solution and of the solution to be tested were put into a test tube and heated in a boiling water bath for 15 minutes. For the quantitative determination of the amount of reduction, the Schaffer and Hartman iodometric modification of the Munson and Walker method was used. When much of the product was present, the solution reduced the copper very distinctly in the cold. In all cases some reduction was obtained from all fatty acids tried, after sufficient treatment. Considering the reaction only from the standpoint of the amount of reducing substance produced, there are at least three statements that may be made: (1) That the reaction increased with temperature and time, within limits, as would be expected; (2) that the fatty acids from linseed oil yielded more reducing substance than the neutral oil; (3) that the reducing substance increases with the degree of unsaturation of the fatty acids used. The preliminary fashion in which this phase of the problem was attacked allows only these three points to be made, although several others are fairly evident; it will bear much further work.

The quantitative data available to show the increase of reducing substance with temperature and time (up to a certain limit) appears in table I. In addition to this many estimates made in the test tube in a standard fashion, and carefully estimated, support these figures substantially.

Inasmuch as these determinations were incidental to the main trend of the research they were not carried out in duplicate; they are therefore not

TABLE I

A. EFFECT OF TEMPERATURE RISE ON YIELD FROM MIXED LINSEED FATTY ACIDS

TEMPERATURE °C.	DURATION OF TREATMENT	MGMS. OF COPPER REDUCED PER 100 ML.
Room = 23–25	2 weeks, shaken twice daily. No other mixing.	Distinct trace
40	2 weeks, shaken twice daily. No other mixing.	11.5
65	30 hours, mixed by air	42.0
80	30 hours, mixed by air	48.0
95	30 hours, mixed by air	47.6

B. EFFECT OF TIME OF TREATMENT, TEMPERATURE CONSTANT

95	20 hours, mixed by air	45.0
95	30 hours, mixed by air	47.6
95	72 hours, mixed by air	47.0
95	90 hours, mixed by air	44.0

published as absolutely exact, but the estimates made in the test tube bear out the general trend beyond question. In addition to these data, rough estimates made at intervals of 2 hours with temperature constant at 95° C., showed an increase up to 20–22 hours, after which the amount of reducing substance remained fairly constant. It became apparent that, after the point was reached at which about 45 to 48 mgms. of Cu. would be reduced per 100 ml. of solution, the concentration of reducing substance was not increased. It has not yet been determined what the limiting factor is, although it is likely to be volatility, since part of the reducing substance distills over at 100° C., and the current of air together with the high temperature carries over much volatile material.

TABLE II

COMPARISON OF YIELD OF REDUCING SUBSTANCES BY NEUTRAL LINSEED OIL WITH LINSEED FATTY ACIDS

SUBSTANCE	TEMP. ° C.	TIME	MLS. OF N NaOH USED TO NEUTRALIZE 25 ML. SOLUTION	MGMS. CU REDUCED PER 100 ML.
1. Neutral oil	95	20 hours	0.3	6.6
2. Fatty acids	95	20 hours	1.1	45.2
3. Fatty acids	95	90 hours	1.67	44.0

The second statement, that the free fatty acids of linseed oil yielded more reducing substances than the same amount of neutral oil, is based upon the figures in table II.

It may also be seen from this table that in cases 1 and 2 the greater yield of reducing substances is accompanied by a greater production of acidity of the solution. This is a general phenomenon in these experiments, until after the maximum concentration of the reducing substance is reached; thereafter the acidity increases slowly while the reducing power does not. Case 3 in table II illustrates this. After 90 hours at the same temperature it had no more reducing action, but had more acidity.

Thirdly, it was stated that the greater the degree of unsaturation of the fatty acids, the greater the yield of reducing substances under the same conditions. This is illustrated for several fatty acids in table III.

TABLE III

RELATION BETWEEN UNSATURATION AND YIELD

No.	FATTY ACIDS	IODINE VAL.	TEMP. ° C.	TIME IN HOURS	MGMS. CU PER 100 ML. SOLUTION
1	Linseed fatty acids	170.8	80	30	48.0
2	Cottonseed fatty acids	109.4	80	30	26.0
3	Stearic acid (E & A)	10.4	85	90	10.6

When time is considered the results here show striking differences between fatty acids having different degrees of saturation. Oleic acid belongs between stearic and cottonseed fatty acids, as shown by test tube estimates of reducing power. A purer grade of stearic acid was obtained and used in other experiments, but no quantitative measurement of the amount of reducing compounds yielded was made on this. It had an iodine value of 1.22, yet it still furnished reducing substances under this treatment, though in smaller quantities, as measured by the estimation of the precipitate in the test tube.

The main object of these studies, however, was to find out how close to the carbohydrate condition the products of this mild oxidation approached. One of the chief tests for simple carbohydrates was satisfied in the reduction of copper; the substances also reduced ammoniacal silver, without heating. Further, they gave a positive aldehyde test with the fuchsin-SO_2 test, gave a negative ketone and positive aldehyde reaction with thiobarbituric acid, and positive aldehyde with iodoform and nitroprusside tests. It seemed necessary, then, to conclude that we were dealing with water-soluble aldehydes. It seemed probable since they were water soluble that

they were not long chain fatty aldehydes, *i.e.*, longer than nonylic aldehyde; and since they lacked the strong characteristic odors, they could not be short chain fatty aldehydes. The assumption was made then that the reducing compounds had more oxygen than that of the carbonyl group. This was in the direction of the carbohydrate structure.

The Molisch test for carbohydrates (α-naphthol) gave neither clear-cut positive nor negative results. Different observers pronounced it a "light" test, a faint reddish-violet color, in this case nearly obscured by a heavy brown smudge.

Also, in all cases with all fatty acids tried, from purified stearic with an iodine value of 1.22 to the most unsaturated, the solution from the treatment was in some degree fermentable with bread yeast. Although this did not mean necessarily that the compounds were carbohydrates, the restricted limits into which this reaction threw them made it seem worth while to test out this phase thoroughly. It was found first, that when the treatment was made without a reflux condenser, there were left reducing substances, but no fermentable compounds. Distillation tests showed that this fermentable fraction was carried over at 94° to 99° C. Furthermore, it was not distillable from neutral or alkaline solution, but passed over when the solution was re-acidified and redistilled. It was evidently an organic acid of short length. There was some evidence to show that this compound loses its fermentability when evaporated to dryness on the steam bath; of course this was done in slightly alkaline solution to prevent loss of the substance by evaporation. After fermentation, iodoform tests were made on the distillate from the fermentate, rendered alkaline for distillation. The object was to see if ethyl alcohol was produced by the fermentation, carbon dioxide having been the only measure used till then. Iodoform precipitate was obtained from this distillate, but it formed in the cold, indicating acetone, isopropyl alcohol, or an allied substance, but not ethyl alcohol. Nitroprusside tests for acetone, however, were negative. An iodoform precipitate was obtained in the cold from the distillate from an alkaline solution of the original unknown solution before fermentation; it was also obtained from the distillate made from this alkaline residue after acidifying and redistilling. Negative nitroprusside tests showed that acetone was not present either. It is evident, therefore, that there is more than one substance yielding iodoform present in the original solution, one an acid, one other at least not acid. To give iodoform, the acid would have to have either a hydroxyl group or a double-bonded oxygen in addition to its carboxyl group. Also, it is probable from the knowledge available on fermentation that an organic acid, to be fermentable by bread yeast, must have additional oxygen in its structure, and probably must be an alpha-hydroxy- or alpha-ketonic-acid.

In this respect the iodoform and fermentation results agree. It would appear then that we have here an hydroxy or ketonic acid of such length that it distills from its water solution near the boiling point of water.

It was found that the fermentable compound (or compounds) was separable from the main part of the aldehydes which furnished the reducing action, by distillation. A large portion of the aldehydes was carried over from slightly alkaline solution which retained the fermentable part. The reducing power was much diminished by prolonged heating, especially in alkaline solution, and the residue readily took on a charred appearance with a crisp physical character. We know, therefore, that the aldehyde portion is not the fermentable substance; that it is in part carried over from its boiling solution, and partly destroyed in the distillation; that it probably contains more oxygen than the fatty aldehydes, since it has not the characteristic strong odors possessed by acetaldehyde, propionic, butyric, heptylic, nonylic, and other fatty aldehydes.

With this mixture, it was obviously difficult to work with the phenylhydrazine reactions. Considerable effort was spent with phenylhydrazine and modified phenylhydrazines, and while some results were obtained, they were not consistent. It was quite clear that none of the osazones of the carbohydrates were obtainable. This line of attack was then left until methods of purification and concentration without destruction should be perfected for this special mixture.

In the endeavor to separate out components of the mixture, their solubility in alcohol and ether was tested. The original solution was evaporated to dryness slowly, and the residue extracted first with ether, second with absolute alcohol, and third with distilled water, all at room temperature. This distilled water extract is designated as S_1. After the three extractions were made considerable residue (R_1) remained. Also, when the ether and alcohol extracts were freed from the ether and alcohol and 20 ml. distilled water added to take up the soluble portions, (S_2 and S_3), considerable remained as residue (R_2 and R_3). To each of the three residues 2 ml. of N NaOH were added; the NaOH dissolved the ether and alcohol residues (R_2 and R_3), and a part of the water extraction residue (R_1). These (S_4, S_5, and S_6) were made up to volume equal to the extractions and all were compared as to reducing action, fermentability, and Molisch test.

The ether extraction removed most of the fermentable substances, although small amounts were left in the alcohol, and in the residue. But none of these substances were taken up by the water, until after the hydroxide was added, showing again that the fermentable portion is an acid. It is likely, too, that the evaporation to dryness causes a change in these acids,

a polymerization of some sort; this is suggested to explain why they are water soluble at the outset but are not after the evaporation. Also, the fact that a resinous type of residue results from evaporation to dryness, suggests polymerization, although, of course, we do not know from that which compounds are affected here.

The ether extract contained about one fourth of the total reducing substances, most of this fourth going into the water added after the ether was removed (S_2). The alcohol extract, following the ether extraction, contained about one tenth of the total reducing power, most of it being water soluble (S_3) again after the alcohol was evaporated off. The remainder of the reducing substances, the greatest part of them, was found in the water extraction (S_1) taken on the original products following the ether and alcohol extractions. A trace was found in the residue left (R_1) after all the extractions, and it was liberated by the addition of the hydroxide. These results were obtained from duplicate attempts made along this line, and were the same in each case. As mentioned above, these extractions were made at room temperature. By shaking ether with the original solution, from one fourth to two thirds (approximately) of the reducing power could be taken up, depending on the way the original solution was prepared. Three washings with fresh ether were made in each case. This again indicated that there were two kinds of reducing substance present. The best Molisch test (for carbohydrates and like compounds) given was obtained from the high reducing fraction, the water extract (S_1). It was positive, but not a strong test. No other of the separations gave the test at all.

The ether extract gave an odor of burnt sugar, combined with a fruity odor. "Maple sugar," "fudge," "burnt candy," etc., were suggested by different individuals who smelled it. It proved to be attractive to flies also. Molds grew on solutions from the ether extract, also on the alcohol extract which contained the fermentable compounds; and care had to be taken with all the original solutions made from any of the fatty acids used, since solutions from all became contaminated with molds upon standing at room temperature.

An attempt was made to get a measure of the length of chain of one of the acids present in the mixture which was carried over from its water solution at the boiling point, by means of its neutralization value. I was not sure of the purity of the compound, and was not sure of its oxygen content. Assuming it to be of the acetic series it would have to be an 8-carbon chain, or more probably a mixture of 7 and 9-carbon chains. However, it is very likely that there were other volatile impurities which would invalidate these figures and that there is more oxygen in the molecule than in the acetic series.

Several compounds were tested for, which had been reported in the literature as coming from the decomposition of fatty acids under certain conditions, different from those used in these experiments, but in general conducing to oxidation of the molecule. Thus, acrolein, acetone, epihydrin aldehyde, pyruvic acid, and lactic acid, were tested for and all were found to be absent from the solution obtained from the oxydation by this method. Certain of the strong smelling fatty aldehydes, such as butyric, heptylic, nonylic, etc., were known to be absent by the absence of the rancid odor they are known to impart to certain old fats.

From these studies it can be seen that much is yet to be done before we can speak with certainty on the manner of breakdown of these fatty acids under these conditions; the conditions are simple—oxygen, water, temperature (mild), and the decomposition of the acids should more likely take place at the weakest points than if the conditions were more drastic. More study of the products must be made, also, but certain statements can be made on the basis of these experiments that will at least serve as suggestions to other workers in this particular phase of fat chemistry. First, we can say that all fatty acids yielded a fermentable acid, not of the acetic series, and not pyruvic nor lactic acid. It is ether- and water-soluble and distils from its water solution, going over with the first fraction. It seems to polymerize readily on drying. Second, these same fatty acids yield with the same treatment water soluble aldehydes, not fermentable with yeast. They reduce Fehling's solution, and give various specific aldehyde tests. Some, at least, can be distilled from their water solution. They tend to char and are easily destroyed. The insolubility of some of these reducing substances in ether indicates the presence of more oxygen than the one atom in the carbonyl group. The fact that they were in great part insoluble in absolute alcohol and yet were carried over in part during distillation of their solution requires further work for explanation. Possibly only the ether soluble and alcohol soluble portion was distillable. It should be mentioned here again that the best Molisch test for carbohydrates obtained at all was given by this water-soluble-only aldehyde substance. This is the point nearest to the carbohydrate group arrived at in these investigations. Third, it is evident that more than one aldehyde was produced, some being aromatic, others (one other at least) not.

Changes in fatty acids during treatment

The fatty acids were found to undergo some very decided changes during the course of these treatments, and with a view to getting more light on the nature of the decomposition reactions, these fatty acids were studied before and after the treatment by the standard methods of examining fats,

viz.: specific gravity, saponification value, neutralization value, lactone value, iodine value, acetyl value, etc. Results from such examinations are shown in table IV.

TABLE IV

DATA ON CHANGES OCCURRING IN FATTY ACIDS DURING TREATMENT

No.	Test	Linseed fatty acids		Stearic acid (E & A)	
		Before	After	Before	After
1	Specific gravity	0.9079	1.0112	0.8902	0.9114
2	Saponification value	194.4	236.3	215.87	223.60
3	Neutralization value	194.4	144.4	204.3	193.1
4	Lactone or anhydride value	1.6	91.9	11.54	30.5
5	Iodine value	170.8	73.5	10.4	2.93
6	Acetyl value	68.03	114.8		

In addition to these changes, the viscosity of the liquid fatty acids increased during treatment. The increased specific gravity resulted in the oil layer being at the bottom of the flask in which the treatment occurred instead of at the top, after sufficient oxidation had taken place. This was found only in the linseed fatty acids. This increase of specific gravity is undoubtedly due to the absorption of oxygen; the decrease of the iodine value bears this out, and the increase of the acetyl value shows that part of this extra oxygen taken on is retained as hydroxyl groups. The increase in the saponification value indicates an increase in the number of carboxyl groups per unit weight, caused by the formation of shorter chain fatty acids. The fact that the neutralization value did not follow the saponification value, and the fact that the lactone value increases so markedly during treatment gives evidence of the formation of lactones or of polymerization products, in either case yielding water and forming an anhydride. This is dependent upon the presence of hydroxylated fatty acids and is further evidence of the formation of these. In fact it is necessary to consider both the acetyl value and the lactone value in getting an estimate of the degree of hydroxylation of the fat, since the formation of the lactones would lower the acetyl value in the same degree in which they were formed.

Although some of the changes produced in the fatty acids are similar to those occurring in rancidity of fats, blowing of oils, and other conditions under which oils have been oxidized, the products are quite different in this case from those obtained in the other methods of treatment. All products reported in the literature as resulting from other methods of oxidation have been tested for, the outstanding ones especially, such as acetone, fatty ketones, acrolein, butyric aldehyde, butyric acid, epihydrin aldehyde,

heptylic and nonylic aldehydes, etc., and have not been found present in detectable quantities in the solutions resulting from this method. It is thought that this method has the virtue of allowing a mild oxidation to occur in water solution (except for the first stages, which of course occur in the fat itself), the water entering into the reaction and yielding a different group of products from those just mentioned. Most experiments along this line have either had no water present or have been too drastic to secure a breakdown along lines that approach the biochemical change we are studying. No doubt pure oxygen, used instead of air for the blowing through the mixture of fatty acids and water, would hasten the oxidation; and perhaps many things may be added to accelerate the reaction, particularly the ordinary mineral catalysts. But these have not been tried in this maiden attempt because it was considered best to solve first the problems brought up by the simple treatment as it was, before introducing new complications of uncertain value. Hastening the oxidation may well change the reaction and the products entirely, and these modifications should, therefore, form another problem for later consideration.

Summary

1. Fatty acids when treated in the manner described in this paper undergo a mild oxidation, in which they absorb oxygen, become hydroxylated, and break up into shorter-chain acids, some soluble in water and some not, and into aldehydes, soluble in water; they probably yield other fragments, too. This breakdown differs in its products from all other reported methods of oxidizing fatty acids, and probably approaches more nearly to the conditions obtaining in the plant, though still far from them.

2. The products remaining in the water solution after this treatment, at any rate approach more nearly the sugars in structure than the products reported in the literature from other methods of oxidation. Water-soluble, ether-insoluble, non-volatile aldehydes which have more oxygen than that of the aldehyde or carbonyl group have been found; the actual structure of these is not known but it is considered an approach toward the carbohydrate condition, and toward the solution of the problem of explaining the common biological interchangeability of the fats and sugars.

3. In addition to these aldehydes there are some that are both water soluble and ether soluble, and are distilled from water solution.

4. Short chain acids are produced which are water soluble, ether and alcohol soluble, can be distilled from their water solution, and are fermentable by bread yeast, yielding carbon dioxide and fermenting in about the same time as glucose with the same yeast. Molds grew on these solutions, but only on the fermentable ones.

Change of the writer to a different field of endeavor not involving laboratory connections is responsible for the appearance of this report of an undertaking that is obviously unfinished. It is made, however, largely for its suggestive value and it is hoped that in this respect at least it will justify its being added to the scientific literature. Chief among those to whom I am indebted for helpful discussion in this work are: Professor F. C. KOCH and Professor C. A. SHULL, of the University of Chicago, Dr. S. H. ECKERSON, Boyce Thompson Institute, and Dr. LOUISA E. RHINE. The generous efforts of Dr. P. D. STRAUSBAUGH in providing time and materials for continuing work at West Virginia University are gratefully acknowledged. The proper citation and discussion of the enormous literature on the various branches of science touched upon in this paper would more than double its size. Reasonable brevity is the excuse for its omission.

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THE DISTRIBUTION OF THE ROOTS OF SUGAR CANE IN THE SOIL IN THE HAWAIIAN ISLANDS

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(WITH SIX FIGURES)

Introduction

Up to the present time several excellent excavation studies of the roots of sugar cane and other crop plants have been made, from which observations of considerable value have been secured. It is generally appreciated, however, that in the biological sciences quantitative determinations are more valuable than statements of observation and for that reason the methods outlined in this paper were developed. These methods with slight modifications are feasible for use with a number of crop plants, so that they may be of interest to other investigators in the plant sciences. An examination of the stimulating studies by WEAVER (**3**), and his associates, in which the literature of root studies has been extensively reviewed, indicates that the methods employed in the present studies have not been previously in use, to any general extent at least.

In growing sugar cane or almost any crop, a large proportion of the cost of producing the crop is chargeable to the operations of tillage in preparation for root growth. It is obvious that this investment in tillage can be more intelligently made if it is known where the roots of the crop will extend or where they may be made to extend. Moreover a knowledge of the distribution of the roots enables one to prevent their injury by cutting or mutilation during the operations of cultivation. Determinations of soil moisture may be confined to the levels of the soil where the roots exist, if it can be determined where the large proportion of the roots become distributed. Irrigation applications can be more economically applied, and water conserved if it is known where the roots exist. The greatest efficiency in the use of fertilizers depends on placing them where they will be quickly available to the greatest number of roots. This is a concise statement of some of the more ready applications of knowledge of root distribution to economical and efficient crop production. WEAVER (**2**) has discussed in greater detail many applications of this knowledge to the growth of plants.

The root-study-box methods

Our first method consisted in growing sugar cane in wooden boxes, thirty by thirty inches square and thirty-six inches deep; in these boxes wire poul-

try netting with a 1½ inch mesh was placed horizontally at different depths and the sides of the boxes were detachable. The boxes were then filled with soil and the cane planted. When the cane reached an age at which it was desirable to make the determinations, the sides were taken from the boxes, the soil was washed away with a stream of water, and the roots were exposed but held in their correct position of growth by the horizontally placed poultry netting. This is more readily understood from the illustration shown in fig. 1 than from the written description. These root-study boxes are somewhat similar to the earthenware cylinders used and described by VENKATRAMAN (1).

With the roots exposed and available for examination in this way, the first attempt to secure a quantitative expression of their distribution was by linear measurement. However, as in several previous attempts at root studies, this attempt to measure the length of the roots was not a success due to the large number of secondary and tertiary roots which made such measurements a tedious and very nearly impossible task. For this reason we adopted the procedure of weighing the roots rather than measuring their length. To do this the roots were cut off at different levels in depth, collected together from a given level, washed, oven-dried, and weighed. The first results obtained by these methods are shown in table I which follows:

TABLE I

DISTRIBUTION OF THE ROOTS OF FIVE-MONTHS-OLD CANE, VARIETY D 1135, IN TWO TYPES OF SOIL. THREE PLANTS AVERAGED FOR EACH SOIL TYPE

DEPTH IN THE SOIL	ROOTS OF ALL PLANTS AVERAGED		ROOTS OF PLANTS IN LOOSE SANDY LOAM		ROOTS OF PLANTS IN COMPACT LOAMY CLAY	
	Grams	Per cent.	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	75.5	63.9	50.4	64.3	100.6	64.6
8 to 16 inches	22.6	19.1	15.7	20.1	29.4	18.9
16 to 22 inches	8.6	7.3	6.3	8.0	10.9	7.0
22 to 30 inches	11.3	9.6	6.0	7.6	14.7	9.5
Totals	118.0	99.9	78.4	100.0	155.6	100.0

Although these results were an advance over the previous lack of quantitative data there was considerable doubt as to their validity for field conditions due to the abnormal aeration of the soil in boxes and the more frequent irrigation and presence of wire netting in the boxes. Moreover the roots of cane could only be studied up to a certain age because of the size of the boxes. For this reason a method for use under field conditions was developed.

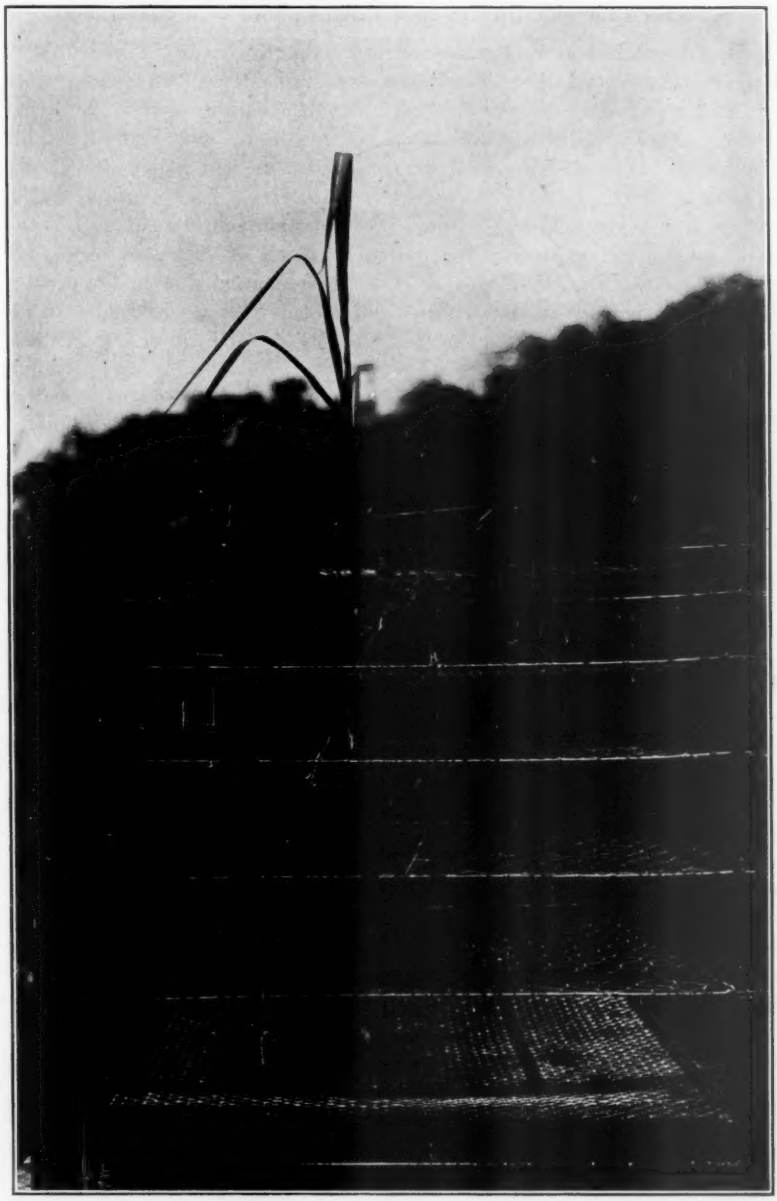

FIG. 1. Root-study box with detachable sides removed and the soil washed away leaving the roots of the plant held in position by the horizontally placed wire netting. Sugar cane, variety H109, one month after planting.

Methods for quantitative root studies under field conditions

In this method as in the method in root-study boxes the principle of weighing the roots rather than linear measurement was resorted to, since dry root weights are fully as good an index of absorbing surfaces as linear measurements when we are dealing with fibrous roots, and the root weights are readily obtained; whereas linear measurements are so difficult to make as to be impractical.

An area in the field was selected in which representative stools of cane could be found for study. The outermost stools of cane in a row were always avoided since they may so easily be abnormal due to the greater amounts of air, light or irrigation water at the edge of the field. In all cases the determinations were made on five stools of cane and the results averaged per stool.

The five stools of cane to be studied having been selected, the cane was cut down to the juncture of the cane stalks with the soil; this point of juncture was arbitrarily called the surface of the soil. Then from the middle of the cane stools a distance was measured out on either side to the centers of the spaces between the rows. Since the cane studied up to the present has been in all cases in five-foot rows, the distance measured from the centers of the stools was 2.5 feet. Stakes were then driven in at these center points between the rows, forming a rectangle, including the five stools to be studied. In order to form a base mark for measurements of the depths of subsequent excavations, light boards were nailed to these corner stakes and brought to a uniform level with the aid of a spirit level.

At the edge of the rectangle to be excavated a wire screen of quarter-inch mesh, such as is used for screening sand for mortar or cement, was placed in a sloping position. Excavation was then started within the rectangle containing the five stools to be studied and was carried to a level which we arbitrarily fixed in these studies at eight inches below the surface of the soil. As the soil was excavated it was thrown on the wire screen standing at one side, and as the soil was sifted through the screen the roots were separated from the soil and were collected in paper bags. When an excavation reached a level of eight inches, and the roots down to that level had been collected and bagged, a further excavation was made to a depth of sixteen inches and the roots between eight and sixteen inches in depth collected and bagged separately. Further excavations were made to levels of 24, 32, and 40 inches below the surface of the soil or as far down as appreciable quantities of roots were found.

When the excavations reached a depth where only negligible quantities of roots were found, each separate collection of roots was washed, oven-dried and weighed. These weighings gave a quantitative expression of the

FIG. 2. The excavation, from different levels of which the roots have been collected. At either end of the pit is shown the base mark from which measurements are taken to establish the different levels in depth in the excavation. At the right is shown the wire mesh screen through which the excavated soil from the different levels is sifted and the roots separated out.

amounts of roots at the different levels in depth in the soil. An illustration of an excavation in progress is shown in fig. 2.

For different purposes, of course, these levels in the soil need not be always eight inches in depth but may be six or ten inches or whatever is found to give results of the greatest value in application. Roots of the plants being studied, which extend beyond the rectangle excavated, are compensated by the roots of the cane in the parallel rows on either side, so that there is only a slight error if any from such a source. It is appreciated, in heavy soils especially, that there is a loss of very small roots through the wire screen of quarter-inch mesh. However, the proportion of error from this source must be very nearly the same at all levels in the soil, therefore this source of error is not regarded as affecting the results seriously.

No cane stubble was included in the weighing; where the roots were dug up attached to the cane stubble, the roots were separated away with a knife and the stubble discarded.

Some results with this method

The first determination with this method was made on the variety H 109, plant cane, 10.5 months old. This cane had had 28 irrigations and had received 275 pounds of nitrogen and 175 pounds of phosphoric acid per

acre, but no potash. The quantities of roots obtained at the different levels in depth of the soil are shown in table II.

TABLE II

FIRST DETERMINATION OF THE QUANTITIES AND DISTRIBUTION OF THE ROOTS OF CANE IN THE SOIL. THE ROOTS STUDIED WERE OF 10.5 MONTHS-OLD PLANT CANE OF THE VARIETY H109

DEPTH IN THE SOIL	AVERAGE WEIGHT OF ROOTS PER STOOL IN GRAMS	PERCENTAGE OF TOTAL ROOTS
Topmost 8 inches	135.23	70.14
8 to 16 inches	43.92	22.78
16 to 24 inches	11.34	5.88
24 to 30 inches	2.29	1.18
Totals	192.78	99.98

These results are shown graphically in the photograph of the root masses from the different levels, reproduced in fig. 3.

One can see that these results check fairly closely with the results from the root-study boxes recorded in table I, that is, although the total root weights per plant varied considerably in the two studies, the percentages of the total roots in the different levels in depth were not very divergent. In both studies unexpectedly high percentages of the roots were found in the upper eight inches of soil and more than ninety per cent. were found in the upper twenty-four inches of soil. It seemed necessary to substantiate these results and build up a considerable mass of evidence to prove or disprove the presence of such a large proportion of the roots in the uppermost layers of soil. In the following tables are presented the results of a number of root excavations and weighings; the results are in every case the averages of five stools of cane.

In table III are shown the root weights of stools of cane of the Yellow Tip variety from two different fields; in the first field the cane averaged 35 tons per acre as compared to 75 tons of cane per acre in the second field. Both fields were unirrigated, 26 months old at the time of the excavations, and had received identical fertilizer treatment.

In the foregoing table one may observe that although the total roots per plant varied in the two fields as much as forty per cent., the percentages of the roots in the different levels of the soil were very nearly identical for the two fields. As in the preceding excavations also a very large proportion of the roots was in the upper eight inches of soil, in both cases being seventy per cent. or more.

FIG. 3. Comparative masses of roots of the variety H109 sugar cane from different levels in depth in the soil. The root mass at the left is from the topmost 8 inches, and root masses of graduating size to the right are from the lower levels in depth, in sequence. The root mass in the topmost eight-inch layer of soil is a lighter brown color than the masses from greater depths, apparently because of the presence of a much larger proportion of secondary feeding roots near the soil surface which are lighter in color than the primary roots.

TABLE III

COMPARISON OF ROOTS OF CANE OF THE YELLOW TIP VARIETY IN A FIELD WHICH YIELDED 35 TONS OF CANE PER ACRE, WITH THE ROOTS OF CANE OF THE SAME VARIETY IN A FIELD WHICH YIELDED 75 TONS OF CANE PER ACRE

DEPTH IN THE SOIL	ROOTS PER STOOL OF CANE YIELDING 35 TONS PER ACRE		ROOTS PER STOOL OF CANE YIELDING 75 TONS PER ACRE	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	72.5	70	107.3	73
8 to 16 inches	20.3	19	26.1	18
16 to 24 inches	8.7	8	11.6	7
24 to 32 inches	2.9	3	2.9	2
Totals	104.4	100	147.9	100

To extend the evidence as to the distribution of the roots of cane in the soil, two further excavations were made on another plantation, as shown in table IV.

A comparison of the root distribution of the two different varieties is not intended in table IV; the results are grouped together merely for economy of space. These results are in close agreement with those of the previous excavations, showing high proportions of the roots in the uppermost layers of the soil. The results with the seven-months-old Yellow Tip cane illustrate a point which will be developed in a subsequent publication, that young plants have higher proportions of roots in the surface layers of soil than plants of greater age; in other words, the center of gravity of the root masses goes lower with increasing age.

TABLE IV

QUANTITIES AND DISTRIBUTION OF THE ROOTS OF 7-MONTHS-OLD YELLOW TIP CANE AND 11-MONTHS-OLD BADILA CANE

DEPTH IN THE SOIL	SEVEN-MONTHS-OLD CANE OF YELLOW TIP VARIETY		ELEVEN-MONTHS-OLD CANE OF BADILA VARIETY	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	26.1	75	31.9	68.75
8 to 16 inches	5.8	17	8.7	18.75
16 to 32 inches	2.9	8	5.8	12.50
Totals	34.8	100	46.4	100.00

Two additional excavations at different plantations, although they afford no direct comparison of roots in different soil types, still are in agreement with a thesis which has been developed in these studies: That, in general, larger proportions of the roots of sugar cane extend to the lower levels in a soil of a sandy loose texture than in more compact loam or clay soils. The results of these two excavations are shown in table V.

TABLE V

QUANTITIES AND DISTRIBUTION OF ROOTS OF 16-MONTHS-OLD CANE IN HEAVY ADOBE SOIL, AND 11.5-MONTHS-OLD CANE IN LOOSE SANDY LOAM, VARIETY H109, BOTH UNDER IRRIGATION

DEPTH IN THE SOIL	16-MONTHS-OLD H109 IN HEAVY ADOBE SOIL		11.5-MONTHS-OLD H109 IN LOOSE SANDY LOAM	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows			25.82	3.90
0 to 8 inches	44.02	75.5	61.87	54.96
8 to 16 inches	11.06	18.9	33.79	22.68
16 to 24 inches	3.17	5.4	13.86	9.30
24 to 32 inches			7.30	4.90
32 to 40 inches			6.31	4.23
Totals	58.25	99.8	148.95	99.97

This comparison does not in itself prove that sugar cane is more deep-rooted in sandy soil than in a more compact clay soil, but in conjunction with other experience with root excavations we believe that there is such a correlation. Our feeling is that it is the factor of aeration in a sandy soil more than anything else which promotes this deep rooting; but this is merely a conjecture which may lead to other interesting experiments.

A still closer comparison is made of roots on two different soil types from two subsequent excavations on the same plantation; both excavations were of roots of irrigated H 109 of very nearly the same age and fertilizer treatment. However the comparison of the roots in the two different soil types was not free from other variables since the crop in the sandy loam was second-ratoon cane while the crop in the semi-adobe was plant cane. The results are given in table VI.

TABLE VI

QUANTITIES AND DISTRIBUTION OF THE ROOTS OF 21-MONTHS-OLD SECOND RATOONS IN A LOOSE ALLUVIAL SILT AS COMPARED WITH 22-MONTHS-OLD PLANT CANE IN A SEMI-ADOBE, VARIETY H109

DEPTH IN THE SOIL	22-MONTHS-OLD H-109 IN SEMI-ADOBE SOIL		21-MONTHS-OLD H109 IN LOOSE ALLUVIAL SILT	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows	9.58	5.43	3.57	2.20
0 to 2 inches	47.66	27.00	28.94	17.86
2 to 8 inches	93.02	52.70	68.09	42.03
8 to 16 inches	19.36	10.97	38.04	23.48
16 to 24 inches	4.36	2.49	14.38	8.87
24 to 32 inches	1.92	1.08	7.18	4.43
32 to 40 inches	.58	.03	1.76	.98
Totals	176.48	99.70	161.96	99.85

The cane in the loose alluvial silt had a notably larger proportion of roots in the lower levels of soil than the cane in the semi-adobe, yet even in the loose alluvial silt 62 per cent. of the roots were above the 8-inch level. Notwithstanding the fact that the cane in loose alluvial soil was a ratoon crop, and the cane in the semi-adobe was a plant crop, our wide experience in these root studies leads us to feel that of the two variables in this comparison the difference in soil character was the more important one. A diagrammatic drawing of the roots of the 21-months-old H 109 ratoons, the weights of which are recorded in table VI, is shown in fig. 4. An interesting point is that in every excavation to date the cane tonnage of the aerial parts has correlated with the root weights. In the above comparison the 176 grams of roots from the semi-adobe soil was associated with 115 tons of cane per acre, while the 161 grams of roots from the loose alluvial silt was associated with 113 tons of cane per acre.

The next excavations undertaken were of three-months-old cane of two different varieties growing under entirely comparable conditions. The results are given in table VII.

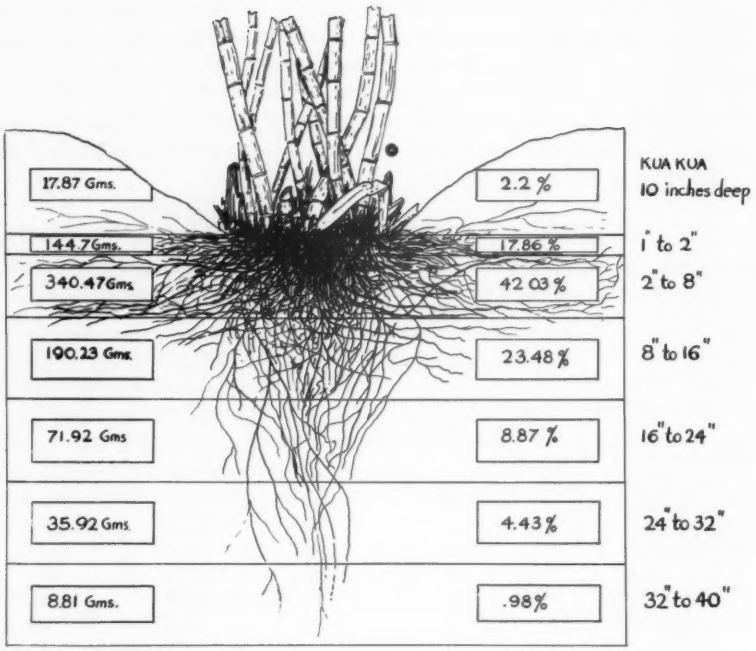

FIG. 4. A diagrammatic drawing prepared from the root weights of 21-months-old H109 sugar cane recorded in table VI. The roots in the lowest levels of the excavations are always found in the center of the pit immediately underneath the aerial parts of the cane plant, giving the root mass something of the shape of a turnip. Drawing by JAMES WILDER and TWIGG SMITH.

TABLE VII

COMPARISON OF THE ROOT QUANTITIES AND DISTRIBUTION OF 3-MONTHS-OLD LAHAINA AND H109 VARIETIES UNDER COMPARABLE CONDITIONS OF ENVIRONMENT

DEPTH IN THE SOIL	3-MONTHS-OLD LAHAINA VARIETY		3-MONTHS-OLD H109 VARIETY	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	13.51	87.86	28.03	88.51
8 to 16 inches	1.74	11.32	3.21	10.13
16 to 24 inches	0.12	.80	0.42	1.35
Totals	15.37	99.98	31.66	99.99

These excavation results show the greater root quantity of the H 109 variety as compared to the Lahaina variety, and also the higher center of gravity in the roots of young cane as compared to cane of greater age. It is noteworthy that although the total roots of the H 109 exceed the total roots of the Lahaina by more than 100 per cent., the percentage distribution of the roots in the different levels of the soil is very nearly identical for the two varieties. As regards the differences in root quantities between the two varieties, Lahaina cane is notably susceptible to several different types of root failure; and the small root quantities tabulated here are probably the result of one of the growth-failure agencies.

Another comparison between the root quantities of the Lahaina and H 109 varieties was possible in cane of nearly the same age, under comparable environmental conditions, and the results are shown in table VIII.

TABLE VIII

COMPARISON OF THE ROOT QUANTITIES AND DISTRIBUTION OF 16-MONTHS-OLD LAHAINA CANE AND 15-MONTHS-OLD H109 UNDER COMPARABLE CONDITIONS OF ENVIRONMENT; BOTH VARIETIES WERE RATOON CROPS, GROWING ON THE RIDGES RATHER THAN IN THE FURROWS

DEPTH IN THE SOIL	16-MONTHS-OLD LAHAINA RATOONS		15-MONTHS-OLD H109 RATOONS	
	Grams	Per cent.	Grams	Per cent.
0 to 8 inches	70.85	19.09	48.14	12.86
8 to 16 inches	159.85	43.08	176.90	47.24
16 to 24 inches	86.97	23.42	94.98	25.36
24 to 32 inches	41.38	11.15	43.48	11.61
32 to 40 inches	12.05	3.24	10.96	2.92
Totals	371.10	99.98	374.46	99.99

These results show no appreciable differences in either root quantities or distribution for these two varieties. The large quantities of roots per stool is due to the fact that in the fields where these studies were made, the stools were much farther apart than is usually the case in most fields. Figured out on the basis of roots per volume of soil the quantities of roots in the studies recorded in table VIII were not unusual. In the Hawaiian Islands there are two distinct types of practice in raising sugar cane; one practice is to grow the cane in furrows and the other is to hill up the cane so that it is growing on the ridges. In the case of the cane for which the root studies are recorded in table VIII, the practice had been to hill up the cane in contrast to the cane in all previous studies where the plants had been growing in the furrows. The results from the hilled-up cane are markedly different

FIG. 5. Root masses of 16-months-old cane of the Lahaina variety at different levels in depth in the soil. The root masses from left to right are collections from the 8 inch soil levels in sequence from the topmost stratum downwards. The outstanding variable influencing the root distribution of the cane in this particular study was the hilling-up of this cane while the cane whose roots are shown in fig. 3 was growing in furrows.

from the root results from the cane in furrows; whereas in the cane in furrows the largest proportion of the roots was found in the topmost eight inches, in the hilled-up cane the largest proportion was found in the level from eight to sixteen inches in depth. A photograph showing graphically the root masses of the 16-months-old Lahaina cane at the different levels in the soil is reproduced in fig. 5. In the hilled-up cane, however, as in the cane in furrows the quantity of roots per volume of soil was greatest in the topmost eight inches. A diagrammatic drawing of the root distribution of the hilled-up H 109 cane, the root weights of which are recorded in table VIII, is reproduced in fig. 6. In both methods of cultivation, excavation studies to date have shown in all cases more than 85 per cent. of the roots existing above the 24-inch level and a large part of the results have shown even higher proportions of the roots in the topmost two feet.

There was one further comparison made of root quantities and distribution, the results of which are seen in table IX. Both varieties in this comparison were growing in a loose sandy soil.

The variety D 1135 is often grown under unirrigated conditions and is generally regarded as more drouth resistant than most sugar cane varieties. In such a connection it is interesting to note that in the foregoing excavations not only are larger proportions of the D 1135 roots in the deeper levels of the soil but there is also a much greater total quantity. The subject of the ratio of roots to aerial parts in connection with drouth resistance in sugar cane will be developed in a subsequent paper. In the foregoing excavation studies as in those previous, more than 85 per cent. of the total roots were in the topmost 24 inches of soil. With the results recorded in this paper from the sixteen different excavations under extremely diverse conditions of environment, each result being the average of five plants,

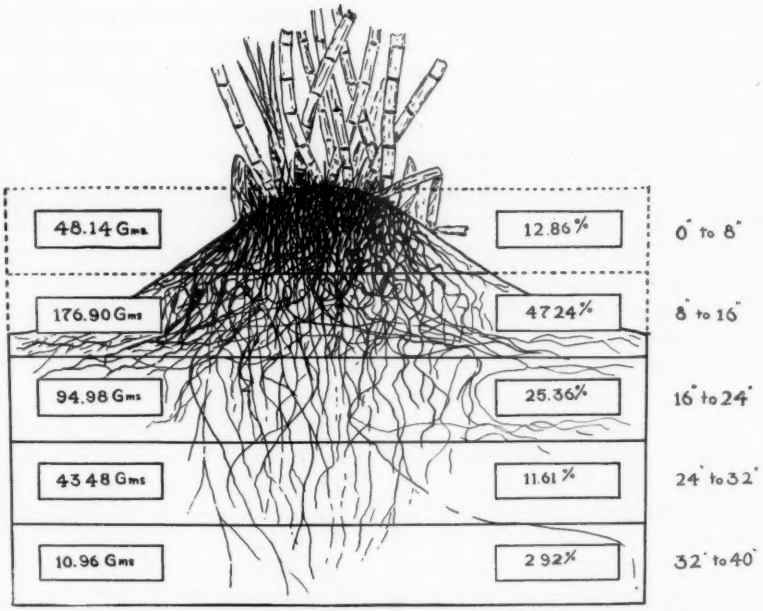

FIG. 6. Diagrammatic drawing by JAMES WILDER made from the root weights of the sugar cane variety H109 recorded in table VIII. This cane was hilled-up as contrasted with the cane illustrated in fig. 4 which was growing in furrows.

TABLE IX

COMPARISON OF THE QUALITIES AND DISTRIBUTION OF THE ROOTS OF THE D1135 AND H109 VARIETIES; BOTH VARIETIES WERE 12-MONTHS-OLD AND GROWN UNDER COMPARABLE CONDITIONS

DEPTH IN THE SOIL	D1135		H109	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows	18.83	10.25	4.44	3.65
0 to 8 inches	89.73	48.85	72.62	59.81
8 to 16 inches	34.02	18.52	29.54	24.33
16 to 24 inches	21.63	11.77	5.15	4.24
24 to 32 inches	13.14	7.15	5.44	4.48
32 to 40 inches	6.30	3.43	4.22	3.47
Totals	183.65	99.97	121.41	99.98

there is recorded a sufficient mass of evidence to indicate that a large proportion of the roots of sugar cane under Hawaiian conditions exist in the

uppermost levels of soil. In none of the foregoing studies have we found as high as 15 per cent. of the roots below the 24-inch level. These methods and results have led to other modes of attack and lines of experimentation which will be presented in subsequent publications.

Some applications of the results

It will be obvious to investigators of the soil environment of sugar cane that soil samples for chemical or physical analyses, to have the greatest application, will be from the layers in depth where the greatest proportion of roots occur. It seems safe to disregard sampling below the 48-inch level at the most, and many investigators will feel that in most cases samples will yield the most valuable information if taken from the topmost eight inches, and from the level 16 to 24 inches in depth. Samples for soil moisture will also be of the greatest value from those strata.

Plantation men have already suggested that these results may indicate that the same quantity of irrigation water applied in several frequent applications would be of greater value than a single application in an effort to secure deep penetration of the water. If one studies carefully the diagrammatic drawing of the roots of the cane in furrows, shown in fig. 4, in comparison with the drawing of the roots of the hilled-up cane, shown in fig. 6, it is apparent that irrigation water, and soluble fertilizers applied in the irrigation water, will reach a much larger proportion of the roots of cane in furrows than of the hilled-up cane where the irrigation water runs between the rows.

On the other hand, if one is growing a cane variety susceptible to root rots or if the cane is to be unirrigated, the roots of the cane on the ridges will secure much better aeration and develop greater feeding surfaces than the roots of the cane in furrows, where the blanket of soil between the rows shuts off aeration to some extent.

In excavating the roots in the foregoing studies it has been observed in many instances that the root masses from the topmost layers will be a considerably lighter color, a light brown, than the root masses from the lower levels of soil which are dead black. This difference in color seems to be due to the much greater number of small secondary feeding roots in the root masses from the topmost layers than in the lower layers of soil. It is also observable that there is a considerably greater amount of cortex rots on the roots from the lower levels of soil than on the roots from near the surface. These observations have led to the feeling, admittedly unsupported by quantitative data, that aeration is the chief factor in developing secondary feeding roots and in preventing cortex and probably stele rots.

The quantitative data presented in the foregoing tables indicates the necessity of shallow cultivation to avoid cutting the roots. Mr. FRANK BROADBENT, Agriculturist of the Hawaiian Commercial and Sugar Company, has pointed out the heavy exudation of the plant juices from the cut end of cane roots and this has since been observed very commonly in these excavation studies. On the other hand one feels that if aeration is the chief factor in promoting the maintenance of good feeding surfaces on the roots, then deep tillage preparatory to planting, and the incorporation of organic matter in the lower levels of the soil, are extremely important.

Sugar cane in the Hawaiian Islands is grown with almost no rotation of the fields to other crops and with but slightly more fallowing. This has led to the question as to how long the crop may be continued in the absence of such practices without injury to the yields. The frequent answer to this has been that the roots of the cane crops provide a quantity of organic matter which remains in the soil and so lessens the need for rotation and fallowing. There have been no quantitative data of the amounts of organic matter returned to the soil by such roots, so that the question has admittedly been an open one. Based on 8,500 stools of cane per acre, found on most irrigated plantations in Hawaii, the root quantities found per stool and reported upon in this paper mean that from 0.9 to 1.85 of a ton of dry organic matter is returned to the soil by the cane roots per crop. The writer would not attempt to say whether this is sufficient to perpetuate the cane crop, but the data at least give a definite basis for discussion and experimentation.

Summary

1. A method was developed for growing sugar cane in boxes which had detachable sides and horizontally placed wire screens; the soil could be washed from these boxes with a stream of water, leaving the roots exposed and held in their natural position by the screens of wire netting. By cutting the roots at given levels, washing them, and weighing them oven-dried, a quantitative knowledge of the distribution of the roots was available. This method was superseded by a method for studying roots under field conditions, but the box method is still useful to supplement the field method.

2. A method of studying the distribution of plants growing under field conditions was developed from the root-study-box method. An excavation was made to the middle points between the cane rows on either side of the plants being studied and to a depth which was made, for convenience, eight inches. As the soil was excavated it was thrown on a wire screen of quarter-inch mesh and as the soil was sifted through the screen the cane roots from the excavated area were separated out and collected in bags.

When the excavation of the first eight inches was completed the excavation was pushed eight inches deeper and the roots from that area collected separately. This collection of roots was continued to lower levels in the soil until only negligible quantities of roots were secured. The roots of the plants being studied which extended beyond the sides of the excavation were compensated by the roots of the plants in the rows adjoining, so that the error from that source was small.

The roots collected from the different levels in depth in the soil were washed, oven-dried and weighed, and the weights of the roots seem to be as good an expression of root surfaces as linear measurements which are much more difficult to secure. The roots of five plants were excavated at a time and the results averaged per plant.

3. Sixteen excavations of five plants each under very divergent conditions of environment are reported in this paper. In the cane growing in furrows a very large proportion of the roots was found in the topmost eight inches of the soil; in every case studied more than 58 per cent. of the roots have been above the eight-inch level in the soil. In the hilled-up cane the largest proportion of the roots, in the cases studied to date, was found in the level from eight to sixteen inches in depth in the soil. In cane, either hilled-up or in furrows, more than 85 per cent. of the roots were found in the topmost 24 inches.

4. The color of the root masses from the uppermost levels in the soil was observed in many cases to be light brown, while that of the root masses from the lower levels in the soil was a darker brown or even black. This led to the observation that there were many more secondary roots with actively feeding surfaces in the root masses from the upper levels of soil than in the masses from greater depths in the soil and these secondary roots gave the lighter brown color to root masses near the soil surface. Rots of the root cortex were also more general in the lower depths of the soil and these rots gave a darker color to the root masses from deep in the soil.

5. A few applications of the knowledge gained in these studies are mentioned.

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THE DIGESTION OF PECTIN AND METHYLATED GLUCOSES BY VARIOUS ORGANISMS*

HAROLD W. COLES

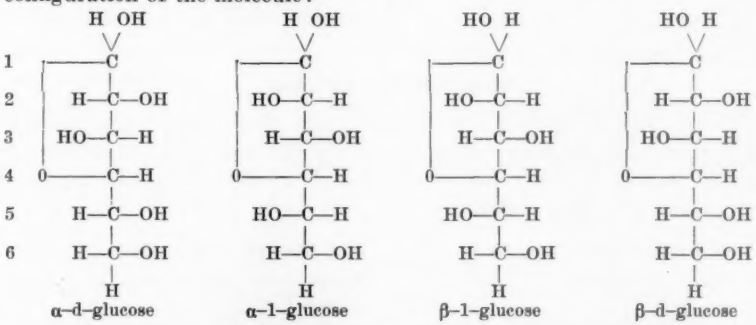
Introduction

This preliminary paper is the result of work started with the object of determining the effect of introduced methyl groups upon the digestion of carbohydrates by microorganisms. At the same time, it was hoped that methylated glucoses and pectin might offer a means of distinguishing between *Bact. aertrycke, Bact. schotmülleri,* and other closely related forms. These two organisms, up to the present time, are not distinguishable on the basis of morphological, cultural, or physiological characteristics. It was also thought that possibly a relation might exist between the digestion of pectin and the methylated glucoses.

The only paper in the literature recording the digestion of methylated hexoses by organisms is that of IRVINE and HOGG (**4**) who record a few experiments made with 3-monomethyl glucose. It was found by these authors that living top yeast and macerated extract, prepared from dried Munich bottom yeast, had no action upon the sugar. They also found that, of the seven species of bacteria tried, *Bacterium coli commune, Bact. lactis aerogenes, Bact. proteus, Bact. paratyphosum, Bact. cloacae, Bact. typhosum,* and *Staphylococcus pyogenes aureus,* all of which are glucose fermenters, only *Bact. cloacae* Jordan digested the methylated glucose with the production of acid and gas.

Methylated glucoses

Glucose may have the following structures[1] depending upon the spacial configuration of the molecule:



* Reported at the meeting of the Iowa Academy of Sciences, April, 1926.

[1] The recent paper of HIRST, Jour. Chem. Soc., London **129**: 350. 1926, indicates that the oxide linkage of glucose should be amylene oxidic <1, 5> instead of butylene oxidic <1, 4> as shown.

It is seen that we might theoretically have methoxy groups attached to carbons one to six on each of the configurations given. Furthermore, we might have all possible combinations of two to five methyl groups for each configuration, and if we include the so-called γ-sugars (compounds in which the oxidic linkage is believed to be other than butylene oxidic <1, 4>) the list is greatly increased.

A simple calculation will show, then, that we have theoretically possible the following methylated glucoses: 20 monomethyl glucoses (including the methyl glucosides), 56 dimethyl glucoses, 76 trimethyl glucoses, 52 tetramethyl glucoses, and 20 pentamethyl glucoses, a total of 224 different methylated glucoses, not including the gamma sugars, since each glucose derivative can occur as the α- or β-modification and each of these as dextro- or levo-glucose.

A careful search of the literature revealed that only a very few of these theoretically possible sugars have been prepared. These are tabulated below, the doubtful ones being indicated by a question mark.

TABLE I

SUMMARY OF METHYLATED GLUCOSES DESCRIBED IN THE LITERATURE

NUMBER OF METHYL GROUPS				
1	2	3	4	5
α-d-1 β-d-1 α-d-2 (?) α-d-3 β-d-3 α-d-6 (?)	α-d-1, 3 α-d-2, 3 β-d-2, 3 α-d-2, 5 (?)	α-d-1, 2, 3, α-d-1, 2, 5, (?) α-d-2, 3, 5, α-d-2, 3, 6, α-d-3, 5, 6, α-d-2, 5, 6, (?)	α-d-1, 2, 3, 5 β-d-1, 2, 3, 5 α-d-1, 2, 3, 6 β-d-1, 2, 3, 6 α-d-1, 4, 5, 6 (?) β-d-2, 3, 5, 6 α-d-2, 3, 5, 6 α-d-3, 4, 5, 6	α-d-1, 2, 3 5, 6 β-d-1, 2, 3 5, 6

Materials and methods

The media used in these investigations were of two kinds: One containing peptone, and the other containing no peptone, and hence no carbon except the carbon of the pectin or the carbon of the methylated glucoses. They were made up as follows:

MEDIUM WITH PEPTONE	MEDIUM WITHOUT PEPTONE
2 gms. bacto-peptone	2 gms. ammonium chloride
1 gm. K_2HPO_4	2 gms. K_2HPO_4
10 cc. 1 per cent. Andrades indicator	10 cc. 1 per cent. Andrades indicator
3 gms. pectin or sugar	3 gms. pectin or sugar
1000 cc. distilled water	1000 cc. distilled water

The media were adjusted to a P_H of 7.0 to 7.2 and tubed. In all of the work, twenty-four hour glucose-phosphate agar slants were used for inoculation of the culture media. Inoculations in every case were made as heavy as possible, care being especially taken with the medium containing no peptone to see that the inoculations were positive. The tubes, after inoculation, were incubated 24 to 98 hours at 37° C., excepting those organisms whose optimum was 25° to 27° C., and any acid, gas or gumminess recorded each day.

The pectin, a very pure form, was obtained from the Research Laboratories of the California Fruit Growers Exchange, and was used as received. It contained, as reported by them, aluminium oxide as an impurity. It was tested for reducing sugar which was found to be absent.

The method followed for the preparation of the 3-monomethyl glucose was essentially that of IRVINE and HOGG (**4**). The reactions involved are:

$$\alpha\text{-d-glucose} \xrightarrow[\text{1 per cent. HCl gas}]{\text{acetone}} \text{diacetone glucose} \xrightarrow[\text{Reference (2)}]{\text{sodium}} \text{Na salt of diacetone glucose} \xrightarrow[\text{iodide}]{\text{methyl}} \text{3-monomethyl diacetone glucose}$$

$$\xrightarrow[\text{acid}]{\text{dilute}} \underset{\text{3-monomethyl glucose (I).}}{\text{CHOH.CHOH.CHOCH}_3\text{.CH.CHOH.CH}_2\text{OH}}$$

The 3-monomethyl glucose thus secured is readily soluble in water, less so in methyl alcohol, and is very sparingly soluble in other organic solvents. It has a melting point of 157–158° C., and a rotation in water of +55.5° (final). It exhibits mutarotation. It was purified by recrystallization from methyl alcohol.

The preparation of 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose may be described together, since they are formed in the same methylation process. The method of HAWORTH (**3**) was used. The reaction occurs as follows:

$$\alpha\text{-d-glucose} \xrightarrow[\text{.25 per cent. HCl}\ \text{Reference (1)}]{\text{CH}_3\text{OH}} \alpha\text{-methyl glucoside} \xrightarrow[\text{NaOH}]{\text{dimethyl sulfate}}$$

$$\underset{\text{pentamethyl glucose (II)}}{\text{CHOCH}_3\text{.CHOCH}_3\text{.CHOCH}_3\text{.CH.CHOCH}_3\text{.CH}_2\text{OCH}_3}$$

and

$$\underset{\text{tetramethyl glucose (III)}}{\overset{\text{CHOCH}_3.\text{CHOCH}_3.\text{CHOCH}_3.\text{CH.CHOCH}_3.\text{CH}_2\text{OH}}{\underset{\text{O}}{\text{---------}}}}$$

The methylated glucoses thus secured are syrups. They were repeatedly fractionated for purification. They did not reduce Fehling's solution.

The tetramethyl glucose (III) is soluble in water, chloroform, methyl iodide and in common organic solvents. It has a refractive index number equal to 1.4583 and $D\ \frac{20}{4}$ equal to 1.158. It does not show mutarotation.

The pentamethyl glucose (II) is soluble in alcohol, water, acetone, ether, and methyl iodide. Index of refraction, n_D is equal to 1.4454. It has the following rotations: + 147.4° in water and + 153.9° in alcohol. It does not show mutarotation.

Description of organisms

The organisms of group I belong to the colon-typhoid group and were mainly of fecal origin. Those of group II were obtained from activated sludge from creamery wastes, and group III are organisms from miscellaneous sources. The figures in parentheses indicate the number of strains used. All cultures which attacked pectin and the methylated glucoses were examined morphologically and culturally to determine their position in the classification of the colon-typhoid group as given by WELDIN and LEVINE (5).

GROUP I

Bacterium coli (4), *Bact. paragrünthali* (2), *Bact. communior* (1), *Bact. coscoroba* (2), *Bact. grünthali* (5), *Bact. neapolitanum* (1), *Bact. pseudocoloides* (1), *Bact. schafferi* (4), *Bact. vesiculosum* (2), *Bact. aerogenes* (9), *Bact. cloacae* (7), *Bact. levans* (3), *Bact. oxytocum* (13), *Bact. vulgaris* (6), *Bact. abortivoequinum* (2), *Bact. aertrycke* (1), *Bact. paratyphi* (5), *Bact. flexneri* (6), *Bact. schotmülleri* (4), *Bact. pullorum* (5), *Bact. morgani* (2), *Bact. enteritidis* (3), *Bact. suipestifer* (3), *Bact. ambiguum* (1), *Bact. typhi murium* (1), *Bact. dispar* (1), *Bact. typhi* (2), *Bact. alkalescens* (1), *Bact. sanguinarium* (2), *Bact. anatum* (1), *Bact. pfaffii* (1), *Bact. jeffersonii* (1), *Bact. rettgeri* (1), *Bact. shigae* (1), *Bact. rhinoscleromatis* (1), *Bact. viscosum* (1), *Bact. viscosum aerogenes* (1).

GROUP II

Bacillus albolactus (2), *B. cereus* (4), *B. mesentericus* (1), *B. panis* (2), *B. megatherium* (1), *B. aterrimus* (1), *B. niger* (1), *Pseudomonas myxo-*

genes (1), *Pseud. fluorescens* (1), *Pseud. ovalis* (2), *Pseud. pavonacea* (3), *Flavobacterium ovalis* (2), *Flav. acetylicum* (2), *Flav. suaveolens* (3), *Flav. zettnowi* (1), *Flav. deciduosum* (1), *Serratia rubida* (2), *Ser. rubrica* (2), *Ser. amyloruber* (1), *Bact. metalkalescens* (1), *Rhodococcus coralinus* (1).

GROUP III

Saccharomyces ellipsoideus (2), *Sac. cereviseae* (1), *Torula rosea* (1), *B. subtilis* (2), *B. sphaericus* (1), *B. mesentericus* (1), *Staphylococcus aureus* (2), *Staph. albus* (3), *Ser. marcescens* (3), *B. fusiformis* (1), *Pasteurella cholera gallivarum* (1), *B. aceto-ethylicus* (2), *Pseud. cyanogenes* (3), *Pseud. pyocyanea* (2), *Pseud. fluorescens* (1), *Sac. pombe* (1), *Past. suiseptica* (1), *Sarcina lutea* (1), *Monilia variabilis* (1), *Micrococcus luteus* (1), *B. mesentericus fuscus* (1), *Bact. abortus* (1), *Micr. flavescens* (1).

Results

The cultures which attacked the pectin with the production of acid and gas are the following, the numbers in parentheses being the laboratory numbers of the cultures: Seven out of thirteen strains of *Bact. oxytocum* (305, 369, 499, 139, 261, 269, 270); four out of nine strains of *Bact. aerogenes* (117, 80, 256, 257); two out of two strains of *Bact. viscosum aerogenes* (248, 298); and two out of two strains of *Bact. aceto-ethylicus* (172, 173). Garden soil and manure produced acid and no gas, and garden soil produced gumminess in both media. *Bact. schafferi* (103) produced gumminess in peptone media. All other cultures of Groups 1 to 3 failed to attack pectin with the production of acid, gas or gumminess. There was no difference between the two media, *i.e.*, no culture produced acid or gas in the synthetic non-peptone medium which did not produce it in the peptone medium, and vice versa.

A larger variety of cultures produced acid and gas from the 3-monomethyl glucose. They were: Six out of seven strains of *Bact. cloacae* (233, 235, 236, 264, 136, 263); one out of three strains of *Bact. levans* (238); one out of fourteen strains of *Bact. oxytocum* (499); one strain of *Bact. pseudocoloides* (486); and one out of nine strains of *Bact. aerogenes* (268). Manure gave acid and gas, but garden soil gave acid and no gas.

The tetramethyl and pentamethyl glucoses were not digested by any of the cultures of Groups 1, 2 or 3 with the production of acid and gas. The test with manure was indefinite, a slight acidity being produced with a bubble of gas.

Discussion

From a consideration of the cultures used and the cultures digesting pectin with the production of acid and gas, it is evident that only those

organisms commonly occurring in the soil are capable of attacking pectin with the production of acid and gas. None of the intestinal forms, classified in the sub-genus *Escherichia* of the genus *Bacterium*, produced acid or gas from the pectin. It was to be expected that organisms digesting pectin would be found in the soil, since the rotting of wood and plant tissue, containing pectin bodies, takes place readily in the soil.

Again, it is evident that only those organisms occurring commonly in the soil are capable of attacking 3-monomethyl-glucose with the production of acid and gas. It was expected that very few organisms, if any, would be able to touch the tetramethyl and pentamethyl glucoses. In these two sugars the hydroxyl groups are protected by methyl groups, and this parallels the cellulose molecule in which hydroxyl groups are presumably bound in ether linkages. It seems, therefore, that those organisms of the colon-typhoid group, which are vigorous fermenters of glucose, are unable to attack methylated glucoses when the number of methyl groups becomes large.

It is interesting to speculate just how the number and position of the methyl groups in the sugar molecule affects the digesting ability of the organisms. It is hoped to make these speculations the basis of a later investigation on methylated hexoses.

Conclusions

1. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by those members of the colon-typhoid group commonly found in feces.

2. The organisms attacking pectin and 3-monomethyl glucose with the production of acid and gas were those generally associated with the soil.

3. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by organisms isolated from the activated sludge of creamery wastes.

4. All of the members of the colon-typhoid group tested were incapable of digesting 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose.

5. *Bact. schotmülleri, Bact. aertrycke* and other closely related forms can not be differentiated on the basis of the digestion of pectin and the methylated glucoses used.

6. It is suggested that an agar medium containing only 3-monomethyl glucose may be useful for the isolation of *Bact. cloacae*.

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GROWTH OF YOUNG WHEAT PLANTS IN AUTO-IRRIGATED SOILS, AS RELATED TO THE WATER-SUPPLYING POWER OF THE SOIL AND TO THE ADJUSTMENT OF THE AUTO-IRRIGATOR

BURTON E. LIVINGSTON, TAKEWO HEMMI AND J. DEAN WILSON

(WITH ONE FIGURE)

Introduction

The auto-irrigator (**4, 5**) furnishes a convenient means for maintaining approximately constant moisture contents in the soil masses of pot cultures, the maintained moisture content being dependent on the kind of soil used (indicated by its water-holding power, moisture equivalent, etc.), on the packing of the soil, and on the hydrostatic head against which water moves from the irrigator reservoir into the soil mass.

The experiments briefly described below were planned as a preliminary study of the relations between the growth of young wheat plants in auto-irrigated pot cultures and the water-supplying power of the soil about their roots, this dynamic soil feature being itself controlled, for each kind of soil used, by the water content of the soil, controlled in turn by the height of the mercury column introduced between the irrigator reservoir and the water-supplying, porous-porcelain cone. The experiment here reported was carried out by HEMMI in the greenhouse of the Laboratory of Plant Physiology of the Johns Hopkins University, in the winter of 1922–23, and he left his notes with the other authors of this paper when he returned to Japan. The results seem worthy of publication because of the great fundamental importance of the water-supplying power of the soil in plant physiology and plant culture, and because they add considerably to our knowledge of the manner in which the auto-irrigator operates in practice. It seems to be becoming more generally appreciated that the influential conditions of plant environments must be controlled or quantitatively recorded for effective experimentation in plant physiology, and the moisture conditions of the soil are now among the environmental conditions most susceptible of control and satisfactory measurement.

Materials and methods

Three different kinds of soils were used (intended to be like numbers 3, 6, and 9 of LIVINGSTON and KOKETSU'S (**6**) series): a half-and-half sand-

loam mixture with a water-holding power of 39.2 per cent., a fertile loam with a water-holding power of 60.7 per cent., and a half-and-half humus-loam mixture with a water-holding power of 95.1 per cent., these values being determined by the Hilgard method and expressed on the basis of dry weight. On the basis of dry volume they are 53.5, 57.5, and 78.3 per cent., respectively. Tinned sheet-iron containers were used as pots, 15 cm. in diameter and 20 cm. high, each furnished with an auto-irrigator, employing the porous-porcelain cone after the manner described by LIVINGSTON in 1918. Between the reservoir and the cone was inserted a glass U-tube containing mercury, to introduce hydrostatic head against which all water passing into the soil mass must move. Five containers were used for each kind of soil, with different heights of mercury column in the U-tube, these heights being 2, 10, 20, 30, and 40 cm. The irrigators were allowed to operate preliminarily until the capillary water system of the soil closely approached equilibrium with the mercury column, as shown by the fact that the soil masses ceased practically to gain in weight. Then five wheat seeds were planted in each container and the plants produced were allowed to develop six weeks. During this period the water-supplying power of the soil, at a depth of 6 centimeters, was determined from time to time by the soil point method (**6, 7**), the results being recorded as milligrams of water absorbed by a single soil point in a two-hour period. (Later work indicates that a one-hour period is better for such determinations, but the results here reported are satisfactory for comparisons within their own series, though they are not comparable with results based upon any other time period.) The standard soil point, of porous porcelain, has an absorbing surface of about 12 sq. cm., and the two-hour indices of water-supplying power here given may consequently be converted so as to refer to a single square centimeter of absorbing surface by dividing each value by 12. No reliable supplying-power value was secured for the soil in equilibrium with the two-centimeter column of mercury in any case, nor was a reliable value secured for loam in equilibrium with the 10 cm. column, these omissions being due to the use of a shorter time period for the soil-point tests in the cases mentioned. The values were very high in all these cases, surely above the absorbing capacity of the soil point for the soils in equilibrium with the two-centimeter column. The omissions do not detract materially from the value of the results, as will be seen later when the numerical data of the experiment are presented.

Determinations of the water content of each container were also made from time to time, by means of small samples removed with an ordinary cork borer with a diameter of about 1 cm., the holes in the soil being immediately filled with fresh soil of the same kind after each sampling. The

water-content values were recorded as percentages of the volume of the soil and also as percentages of its dry weight.

The several indices of water-supplying power for each container were finally averaged, as were also the several indices of water content. At the end of the experiment the tops of the plants were cut off at the soil surface and their green and dry weights determined, as well as their water contents, as indices of growth vigor. These plant values were recorded as averages per plant six weeks old. The auto-irrigators were read from time to time during the experiment and the total amount of water delivered to the soil of each container during the six-week period was secured by summing the readings.

Results

The data secured from the experiment are shown in table I.

The values given in the table are represented by the graphs of the accompanying figure 1, there being a graph for each of the three kinds of soil.

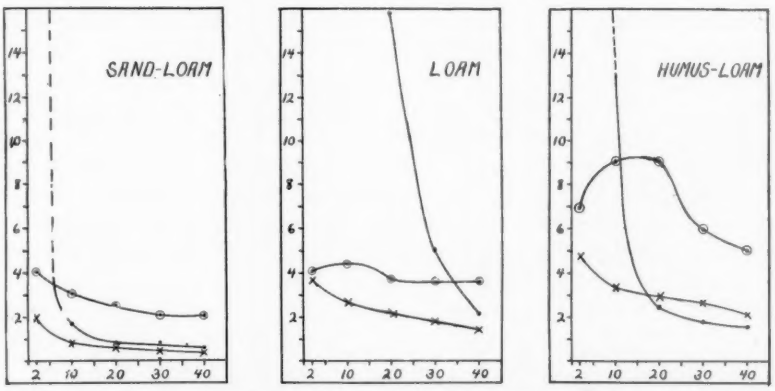

FIG. 1. Graphs of water-supplying power, water content, and plant growth (green weight of tops of six-week wheat plants), as these were related to the mercury column in the auto-irrigator with which the soil was in equilibrium. Three different soils are represented. The steepest curve is for water-supplying power, the curve for water content has crosses at the points, and that for green weight has circles at the points. Abscissas are heights of mercury column in centimeters. Data are from accompanying table.

Abscissas represent heights of the mercury columns, and ordinates represent supplying-power values (for the steepest curve), water content values (for the lowest curve, marked with crosses at the points), and green weights of tops (for the intermediate curve, marked with circles at the points).

TABLE I

WATER-SUPPLYING POWER AND WATER CONTENT OF AUTO-IRRIGATED SOILS IN EQUILIBRIUM WITH DIFFERENT HEIGHTS OF MERCURY COLUMN, TOGETHER WITH GREEN AND DRY WEIGHTS AND WATER CONTENTS OF TOPS OF WHEAT PLANTS SIX WEEKS OLD THAT HAD GROWN IN THESE SOILS

Kind of soil	Total amount of water delivered to soil by irrigator in 6 weeks	Height of mercury column in irrigator	Average index of water-supplying power for 2-hour period of exposure	Average water content of soil		Indices of plant growth		Average water content per plant
				Volumetric	Gravimetric (on dry-weight basis)	Average green weight of tops per plant	Average dry weight of tops per plant	
	cc.	cm.	mg.	Per cent.	Per cent.	mg.	mg.	mg.
Sand-loam mixture	1421	2	.....	20.2	14.2	404	54	350
	643	10	170	7.9	5.3	304	42	262
	347	20	90	6.2	4.2	254	36	218
	191	30	90	4.8	3.3	210	30	180
	161	40	60	4.1	2.8	210	30	180
Loam	1238	2	.....	36.1	28.4	406	46	360
	1294	10	.....	26.8	19.9	441	50	391
	1148	20	1580	22.4	16.0	370	44	326
	940	30	510	17.5	12.5	358	48	310
	740	40	220	14.4	10.6	360	48	312
Humus-loam mixture	1031	2	.....	47.3	56.2	690	82	608
	610	10	1310	33.5	36.1	906	92	814
	473	20	240	29.4	32.1	904	96	808
	268	30	180	26.9	29.4	598	66	532
	225	40	160	21.4	23.3	504	58	446

Discussion

The following observations may be made here, from the data. The water-supplying power consistently follows the water content in the case of each soil, though these two properties are not proportional. For each soil the water content is determined, within limits, by the height of the mercury column. The water content on an auto-irrigated soil mass may have any value between that which is in equilibrium with the highest mercury column that can be used and that corresponding to the maximum capillary content, but its lower limit is fixed by the physical characteristics of the soil employed. For any soil there is a certain range of water contents and corresponding water-supplying powers that can be established and maintained by the auto-irrigator. The upper limit of this range is the maximum capillary content, with the mercury column just balancing the water column between irrigator cone and reservoir. The lower limit is very low for sandy soils, but high for clays and humus soils, with the highest mercury column that can be applied. For the five heights of mercury column used in this experiment the ranges of water content secured were: from 20 to 5 per cent. for the sand-loam mixture, from 36 to 14 per cent. for the loam soil, and from 47 to 21 per cent. for the humus-loam mixture. The corresponding water-supplying powers ranged from very high values (probably well above 2,500) to 60 for the sand-loam mixture, to 220 for the loam soil, and to 160 for the humus-loam mixture. To secure still lower water contents and supplying-powers, it is at once suggested that still higher mercury columns be employed, but the graphs indicate that increased height of mercury column could have very little effect for the sand-loam or humus-loam mixtures, though a higher mercury column would probably lower the values in question considerably for the loam itself. The present set-up of the auto-irrigator is restricted by the fact that mercury columns higher than about 60 or 65 cm. are not practicable.

If adequate liquid strain (**1, 8**) might be developed in the irrigator system the resistance offered to the entrance of water into the soil might be correspondingly increased, but this has not yet been accomplished. Of course a nearly complete atmosphere of opposing pressure might be developed by the use of suction applied in the irrigator reservoir by means of an air pump, but this has not been tried. At best it could generally give resistances corresponding to no more than about 75 cm. of mercury column.

For any height of mercury column in the auto-irrigator the water-supplying power and the water content of the controlled soil were, in general, lowest for the sand-loam mixture and highest for the humus-loam mixture, being intermediate for loam. They are obviously related to the internal surface forces of the several soils, represented by their water-

holding powers. The higher the water-holding power the greater should be the height of the mercury column in the irrigator, to develop and maintain a given water-supplying power in the soil.

With few slight exceptions, the three different growth indices are consistent and consequently only those for green weight of tops are presented in the graphs. For any height of the mercury column the growth of the plants was most vigorous in the humus-loam mixture, least vigorous in the sand-loam mixture, and intermediate in loam. It is notable that the growth values corresponding to the two-centimeter column of mercury are nearly alike for the sand-loam mixture (404) and for loam (406), while the corresponding value for the humus-loam mixture (690) is much greater.

The greatest growth values for the sand-loam mixture were secured with the highest water-supplying power (not measured), with a volumetric water content of 20.2 per cent. and with the shortest mercury column (2 cm.). The twenty-centimeter, thirty-centimeter, and forty-centimeter columns of mercury with the sand-loam mixture were nearly alike as to water-supplying power, water content, and growth index; and all these values were very low. With these mercury columns growth was markedly retarded, but the plants did not succumb, even with the highest mercury column. Growth would probably have ceased altogether with the highest column and the plants would probably have died, if they had been subjected to somewhat more intense conditions of evaporation and sunshine, or even if the cultures had been continued longer, since the water requirement of a plant of course increases as growth progresses, other influential conditions being unchanged.

For the loam soil the highest growth values were secured with the ten-centimeter column of mercury in the irrigator, a slight relative retardation being apparent for the two-centimeter column. This retardation is probably not to be related to water-supplying power, nor directly to water-content, but rather to the oxygen-supplying power (**2, 3**) of the soil, which is necessarily lower with higher water contents. With the exception of the two-centimeter mercury column, every one of the columns with loam gave very much greater growth than did any of the columns with the sand-loam mixture; the forty-centimeter column with loam gave nearly twice as much green weight per plant as did the same column with the sand-loam mixture.

A very pronounced retardation in growth is shown for the two-centimeter column with the humus-loam mixture. With this mixture the highest green-weight values were secured (being about alike) with the ten-centimeter and twenty-centimeter columns, and these growth values are by far the highest of the whole experiment. A water-supplying power of 1,310 gave the highest growth value (ten-centimeter column) but one of 240 gave a value nearly as high (twenty-centimeter column). Supplying powers

much greater than 1,300 were apparently attended by growth retardation for this soil, probably related to deficient oxygen supply, as mentioned in connection with the similar case with loam. The two-centimeter column gave nearly as poor growth as did the thirty-centimeter column. Growth was very good with the highest column, better than with any column for either of the other soils. To retard growth as much in this mixture, and in the loam soil also, as much as it was retarded in the sand-loam mixture with the forty-centimeter column of mercury, would, as is indicated above, require a still higher column and might not be attainable with any column that could be applied without actual liquid strain. Only in our most sandy soil was the lower limit of the supplying-power requirement for good growth apparently approached with any of our mercury columns.

The amounts of water delivered to the soil masses show somewhat the same relations to the heights of the mercury columns and to growth as do the water contents and water-supplying powers. These rates of delivery of water from the irrigator to the soil are of course largely dependent on the rate of evaporation from the soil surface and on the rate of water absorption by the plants.

It is of special interest and importance to call attention to the observation that the growth of the wheat plants in this experiment was clearly and profoundly influenced in some cases by some variable or variables other than the water-supplying power of the soil. With soils having very high water-supplying powers it is probable, as has been mentioned, that growth retardation is brought about by deficiency in the oxygen-supplying power of the soil. This might explain why a water-supplying power of 1,580 (loam with a twenty-centimeter mercury column) gave a green-weight value of but 370, while a water-supplying power of only 160 (humus-loam mixture with a forty-centimeter mercury column) gave a green-weight value of 504. Other considerations would need to be taken up, however, for a rational and general interpretation of such results. For example, a supplying-power of 1,310 (humus-loam mixture with a ten-centimeter mercury column) gave the highest growth value of the entire series (906 for green weight of tops), while a water-supplying power of only 240 (the same mixture with a twenty-centimeter mercury column) gave practically the same growth index (904). Other apparently anomalous cases may be found in our table.

Other chemical conditions of the soil, aside from its oxygen-supplying power, may of course have exerted influence on the growth of the plants. The loam used is known to have been a productive soil, and the two mixtures used were each half loam. It consequently does not appear likely that any of our plants were retarded by inadequacy in the supply of the necessary inorganic salts and ions. That the humus may have introduced special and

perhaps influential chemical conditions is of course possible, but no consistent interpretation of our results seems to emerge from a study of the data with this possibility in mind. It seems likely that we are here dealing with a matter of considerable complexity, one that will require much further study. It may well be that the water-absorbing powers of the root systems were different for the different treatments, with resulting differences in the ratio of water-absorbing power to transpiring power in the plants themselves. Such a suggestion leads to many possibilities, an *a priori* consideration of which would, however, be premature with our present very limited experimental data. At any rate, the problem of the water relations between plant and soil is worthy of much more serious attention than has thus far been devoted to it. The auto-irrigator, the soil point method, and the method for determining the oxygen-supplying power of the soil should all be valuable in the development of this very important portion of the field of plant physiology and physiological ecology.

In spite of the considerations suggested in the last paragraph, it remains clear that the water-supplying power of the soil was, in general, of primary importance in determining the growth of our wheat plants. If the fifteen different treatments are arranged in the descending order of green-weight values it becomes clear that the highest five growth indices are for the range of water-supplying powers from very high to 160 (humus-loam mixture), while the lowest four growth indices are for the range of water-supplying powers from 170 to 60 (sand-loam mixture).

To avoid possible partial misunderstandings or unjustified deductions, it may be mentioned that our data and interpretations refer to a Baltimore greenhouse in the winter months, to wheat plants grown for six weeks from seed, to the three specific soils used, to auto-irrigators of the type employed, and to standard soil points exposed to the soil at a depth of 6 centimeters for a period of two hours. Other plants, other treatments, and other instrumentation may be expected to give in some cases more or less different results; but the general principles here noted may be expected to obtain.

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THE CHEMICAL ANALYSIS OF PLANT TISSUES*

The work of this committee has been undertaken at the request of the executive board of the Society and in response to numerous expressions of interest from the members. In order that the purpose in view may be understood at the outset a statement of the proposed scope of activity seems to be desirable.

Plant physiologists operating in such applied fields of investigation as agronomy, horticulture and pathology have frequently experienced need for examination of their problems and results in terms of chemical composition of tissues. In many cases it is not feasible to employ a specially trained chemist for this purpose. Moreover, the earlier stages of this phase of the work call generally for the comparative results of somewhat routine chemical analyses. These may point the way to refined qualitative examination and more accurate quantitative results, for which the services of an expert will be required. There is considerable recent evidence to support the practicability of advising competent but less skilled workers in the use of general methods of chemical analysis, where comparative results are concerned. It should be recognized clearly, however, that no such recommendations as are here to be proposed can carry any guarantee of infallibility with all materials and in all situations. A reasonable exercise of critical faculties is invariably demanded of those who would attempt the execution of chemical analyses.

It is not the aim of the committee to attempt to treat completely the applications of chemistry to plant analysis. This is done already in a rather complete manner in such compilations as the Methods of Analysis of the Association of Official Agricultural Chemists. The methods described in this book, however, are not intended for research purposes and, like most encyclopaedic treatises, lack detailed relation to the problems of physiology. Materials of physiological interest are recognized as foundational to this report. For the present, attention will be given chiefly to the much used phases of analysis covering carbohydrates and nitrogenous constituents. In this capacity it is hoped to incorporate current improvements, add discussional approaches to the work and, in general, frame recommendations which may serve as more flexible and specialized directions than are extant in published form. In no sense is dictation of procedure to be presumed upon the experienced investigator. On the other hand, it is suggested that the committee serve as a clearing house

* Recommendations of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists.

of information in which the contributions of experienced workers may be made available for those attempting the preliminary application of chemistry to problems of plant physiology.

A stereotyped method cannot be generally applicable to all the varying complexes of factors in different types of tissue. Perhaps the empirical methods of chemical analysis now generally used will not suffice to delineate accurately the metabolic changes of plants. Nevertheless, there remains for the present an initial span of applied chemistry to which general methods are applicable when modified to meet special requirements. The committee will endeavor to render service in this field, presenting its suggestions from time to time in this Journal. Cooperation is being extended by many workers in plant science whose contributions are herein duly acknowledged. The work will be continued in proportion to its apparent usefulness.

In the present section of the recommendations only general principles of certain phases of the analysis will be presented. Subsequent sections will deal more in detail with special topics.

Sampling

In controlled plant production the variability of composition has not received the attention accorded to yield.

Localization of metabolic effects has been stressed by horticulturists.[1] Where such effects are confined to small tissues it becomes difficult to sample even for micro-quantitative analysis. It is generally feasible, however, to deal separately with the various organs of the plant.

Uniformity of size and appearance of individual plants and organs thereof give no certainty of uniform composition. Sufficient material must be taken to compensate variability in the plant or organ involved. The lower limits of this value must be determined experimentally and might profitably be subjected to cooperative testing. Because of the variability mentioned, as well as the requirements of certain analytical processes, it is advisable to analyze duplicate samples. When composition becomes constant with increasing size of the sample, variability has been compensated.

From twenty to one hundred plants or parts thereof, depending upon the number of uncontrolled variables in production, should suffice for a representative sample. In general, it is advisable to select at least ten times the amount of tissue to be taken as a sample for analysis. So far as possible the minimum of dry matter available from controlled plant cultures should be held at 25 grams.

[1] TUFTS, W. P. Proc. Amer. Soc. Hort. Science. 1925: 232–236.

Extraction

It should be recognized that some enzymatic changes proceed rapidly in crushed plant tissues. Expedition and relatively low temperatures[2] thus become important features of this treatment. The requirements probably vary with the species used and the cultural treatment.

When the extract of the fresh plant tissue is desired, this should be accomplished rapidly in a suitable mill with the addition of liberal amounts of distilled water. Plant fibers will not pass through the sieve plates of a meat chopper, but simple corrugated plates, as in the Nixtamal mill[3], are effective. Qualitative tests upon the extract will determine the extent of washing necessary. The proportion of water used in the grinding process should be adjusted to the degree of succulence of the tissue. Such extracts invariably filter with difficulty, on account of colloidal constituents, but may be washed on a thick pad of paper pulp on a Buchner funnel over a vacuum flask. The filtered extract should be heated to boiling immediately to destroy enzymes, adding a slight excess of calcium carbonate to prevent hydrolysis of sugars when the extracts are appreciably acid. As colloidal material flocculates on standing, immediate analysis is preferable to preservation. Separate aliquots should be acidified with a weak acid (acetic) at the boiling point for recovery of soluble proteins.

When the solubility of colloidal materials, particularly of proteins and dextrins, may be neglected, the most generally convenient and common method of extraction is by hot alcohol. This has the advantage, of course, of immediately checking enzymatic changes of composition. Two procedures are in use. In one, sufficient 95 per cent. ethyl alcohol is used to give at least 80 per cent. concentration thereof as diluted by the water of the tissue. This volume of alcohol is heated to boiling, the cut tissue is dropped in and boiling continued for 15 minutes. Sections of the tissue should be relatively thin to favor prompt penetration of the alcohol, 2 to 3 mm. for succulent plants, and less for woody ones. If allowed to cool slowly material insoluble at room temperature will separate and may be strained off. Several washings with 80 per cent. hot alcohol treated in this manner should complete the extraction, the solutes being recovered by evaporation of the extract at low temperature. If aliquots are taken from the alcoholic extract it should be sampled at uniform temperature, as it has a considerable coefficient of expansion.

Another method includes 50 per cent. alcohol as the effective extracting agent. This has the advantage of high solvent power and in some cases has been found as effective as water. The hot extract is preserved

[2] NEWTON, R., BROWN, W. R., and MARTIN, W. M. Plant Physiol., 1: 57–66. 1926.
[3] Enterprise Mfg. Co., Philadelphia.

by increasing the alcohol concentration to about 80 per cent. Colloidal or other material which separates on cooling should be recovered with the insoluble residue. Large Buchner funnels serve conveniently for the percolation and extraction of considerable masses of tissue.

As a general value it may be accepted that somewhat less than 2 liters of water accomplishes practically complete extraction of succulent tissues. The action of hot alcohol seems to be complete at smaller volumes. It has been found that 100 grams of apple spur tissue is exhausted by about 1250 cc. of 80 per cent. alcohol. The alcoholic treatment denaturizes most proteins and renders them insoluble, but all methods of desiccation also seriously reduce the amount of soluble proteins.

The use of calcium carbonate is usually recommended in connection with extraction, chiefly to prevent the inversion of sucrose by organic acids while heating. Some workers have employed ammonia for this purpose, and it should be more effective in penetrating intact cells. This matter deserves experimental attention. In any event, it should be recognized that such additions almost invariably alter the reaction of the plant sap and are liable to disturb the natural distribution of solutes. For the latter reason also, alkalinity of the extract is to be avoided over appreciable time periods. However, an excess of solid calcium carbonate is not objectionable in this sense. To minimize alterations during the recovery of extracts from distinctly acid tissues, it is advisable to conduct a parallel extraction without the use of a neutralizing reagent.

Plant extracts should be analyzed as promptly as possible. Unavoidable delay is more permissible with alcoholic extracts than watery ones, due to the flocculation of colloids in the latter case, as well as the necessary use of preservatives. BURRELL, of Ohio State University, found values for amino and nitrate nitrogen constant in alcoholic extracts for two weeks. Exposure to light should be avoided. The insoluble residue should be oven-dried and ground as fine as feasible for further analysis, preferably to 100 mesh. For this purpose the Dreef pestle mill[4] is unusually satisfactory.

Desiccation

Past investigation of chemical composition in plant tissues have suffered rather generally from failure to consider the effects of the method of desiccation employed. This is especially true of "air drying," where enzymatic changes may become extensive and mask the real composition of the tissues. THOMAS, of Pennsylvania State College, recommends a temperature of 60° C. in vacuo as maintaining with least disturbance the

[4] Formerly stocked by Eimer and Amend, New York.

original composition of the tissue. SPOEHR and CHIBNALL advise that different types of plant tissue are widely variable in their requirements of desiccating treatment. LINK[5] has discussed the factors involved in heat drying. It is not possible to avoid considerable denaturing of proteins by heat drying, and in highly acid tissues sucrose suffers inversion. In general, with masses of tissue too great for treatment in vacuo, drying at 60° C. with forced ventilation minimizes hydrolytic and respiratory changes without caramelizing the sugars. Many types of tissue permit also a preliminary heating at about 100° C. for a matter of 30 minutes to kill the tissue. Other types, rich in colloidal materials, are more resistant to desiccation and require preliminary alcoholic extraction.

Lipoid removal

The necessity of clearing cell walls from fatty and waxy components in order to facilitate extraction and the entrance of watery solvents is rather obvious and well known. To a considerable extent this function is accomplished by the hot alcoholic extraction previously mentioned. Few other lipoid solvents are applicable to fresh, watery tissue and so universal in solvent effect. SANDO, of the U. S. Bureau of Plant Industry, considers alcohol the most efficient solvent for lipoids in the sense that the latter term includes fats, fatty acids, waxes, sterols and phospholipoids. Certainly this extract is of heterogeneous character and has little significance for expression in quantitative terms. It may be further fractionated by extracting the recovered solids with ether.

When dried tissue is to be dealt with, ordinary (ethyl) ether is to be preferred for extraction. The low boiling point and general handling qualities of this solvent have given it general favor. It must be anhydrous and free from alcohol, or sugars and other non-lipoidal constituents will be removed. This form is advertised by manufacturers at a reasonable increase over the cost of commercial ether. It should be possible to obtain it true to specifications. According to SANDO, the lipoidal extraction of dry material is incomplete as compared with the use of alcohol on fresh tissues. The use of ether should leave all carbohydrates and non-lipoidal nitrogenous constituents in the tissue.

Determination of moisture

Regardless of the completeness of removal of water from tissue, as usually stored and handled it acquires the "air dry" state by attaining moisture equilibrium with the contiguous atmosphere. Hence the neces-

[5] LINK, Jour. Amer. Chem. Soc. **45**: 439–447. 1923; **46**: 2044–2050. 1924; **47**: 470–476. 1925.

sity of determining the actual dry weight used for analysis. It is probably sufficient, as a general principle, to employ the same temperature and desiccator service throughout a single investigation or related ones. Where drying is accomplished *in vacuo* a standard value of barometric pressure should be selected for the temperature employed, as the percentage of water remaining in the tissues varies appreciably with moderate differences in pressure. In the relatively rare cases where volatile organic compounds are present in appreciable proportions their recovery in the process of drying the fresh tissue presents, of course, a special requirement.

Expression of results

The common practice of stating composition as percentages of the dry matter may fail to denote changes in the absolute amounts of constituents. In this respect the amount of constituent per plant, or multiple thereof, is advantageous. CHIBNALL[6] stresses the possibility that variations of less abundant constituents may be masked by increases of inert wall material. In this event the basis of fresh weight will have particular value in the statement of results, unless the water content is rather constant. Perhaps the basis of dry matter plus bound water content[7], if generally practicable of determination, will be found more significant. Physiologists are attempting to determine what bases for expressing results will best represent the concentration of metabolic materials in the living tissues under variable conditions. In view of its long-standing usage it seems advisable to retain the expression of percentages in the dry matter, employing also the basis of green weight and such others as appear particularly significant.

This report was organized by W. E. TOTTINGHAM for the Committee.

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[6] Ann. Bot. 37: 511–518. 1923.

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THE USE OF POTASSIUM OXALATE AS A DELEADING REAGENT*

W. E. LOOMIS

Two deleading reagents are recommended in the Association of Official Agricultural Chemists' Methods of Analysis (**1**), anhydrous sodium carbonate, and potassium oxalate. In addition, sodium sulphate, sodium phosphate, and hydrogen sulphide are advocated by various writers for a number of conditions.

SAWYER (**8**) objected to the use of sodium carbonate for deleading because an excess of the reagent gives an alkaline reaction in which precipitated lead salts are redissolved and levulose is destroyed, even at low concentrations. The addition of the carbonate in such a manner as to avoid an excess is a difficult and tedious procedure. Sodium sulphate is more stable but lead sulphate is precipitated slowly in very fine crystals which are filtered out with some difficulty. Additional crystals frequently form in the filtrate if it is allowed to stand. Sodium phosphate and hydrogen sulphide are effective in removing soluble lead but leave the solution acid and are consequently not well adapted for use where reducing and non-reducing sugars are to be determined separately. Potassium oxalate has been advocated by a number of writers. Of these SAWYER stressed the neutrality of the cleared solution and the convenience with which this reagent is used. MEAD and HARRIS (**6**) and EYNON and LANE (**4**) considered the use of potassium oxalate desirable because of its action in precipitating the alkaline earth metals. They found that this group of substances lowered the reducing power of sugar solutions to which they were added.

Experimental results

EFFECT OF THE DELEADING REAGENT UPON THE P_H OF THE CLEARED SOLUTION.—When quantities of 1.25 sp. gr. neutral-lead-acetate solution varying from 1 to 5 cc. were removed from sugar or plant extract solutions by the addition of an excess of potassium oxalate, the filtrate was found in every case to give a reaction color value falling between 6.8 and 7.2 P_H when tested with LA MOTTE standard indicators and compared with the CLARK and LUBS color chart (**3**). When hydrogen sulphide was used as the deleading reagent the acidity of the filtrate varied with the quantity of lead

* The work reported here was done at Cornell University during the tenure of a National Research Council Fellowship in the Biological Sciences.

acetate removed and for 5 cc. of solution gave P_H values as low as 4.8. It should be stated that there was no apparent inversion of the non-reducing sugars in the extracts of green celery leaves when they were deleaded with hydrogen sulphide and boiled for ten minutes to expel the excess of the gas. Celery leaves collected during the day are high in sugars and about 80 per cent. of these are non-reducing. The P_H of the cooled filtrate was found to be 5.2. With sodium carbonate a distinctly alkaline reaction was obtained by adding an excess of salt above that required to precipitate the lead acetate present. If these alkaline solutions were heated or allowed to stand at room temperature there was a change in color, sometimes the development of a distinct odor, and a rapid destruction of reducing sugars.

EFFECT OF AN EXCESS OF POTASSIUM OXALATE UPON THE REDUCTION OF CUPRIC COPPER.—Bureau of Standards dextrose and "Difco" levulose were used in these tests. One gram of Baker's potassium oxalate crystals was added to 50 cc. of sugar solution and reduction carried out under QUISUMBING and THOMAS conditions (7). The cuprous oxide was first weighed directly and its purity was then determined electrolytically by the official sulphuric-nitric acid method. Check clearings in which 1 cc. of neutral lead acetate solution was removed by a moderate excess of oxalate, are included for comparison. All data are an average of triplicate determinations which varied by less than one per cent.

TABLE I

THE EFFECT OF POTASSIUM OXALATE ON THE REDUCTION OF COPPER BY REDUCING SUGAR SOLUTIONS

REDUCING SOLUTION	Cu_2O PRECIPITATED	SUGAR INDICATED	METALLIC COPPER	SUGAR INDICATED	COPPER IN Cu_2O
	mg.	mg.	mg.	mg.	Per cent.
Levulose solution	399.9	183.0	352.5	181.2	88.15
Levulose plus 1 gm. potassium oxalate	400.5	183.2	352.9	181.4	88.11
Levulose plus lead and potassium oxalate	401.3	183.6	353.6	181.7	88.11
Dextrose solution	293.9	131.7	260.1	131.1	88.53
Dextrose plus 1 gm. potassium oxalate	293.7	131.6	260.3	131.2	88.62
Dextrose plus lead and potassium oxalate	294.2	131.8	260.7	131.4	88.61

No indication is given by the data in table I of any effect on copper reduction from the small excess of potassium oxalate required in deleading, or from an excess approximately eight times as great.

THE CO-PRECIPITATION OF REDUCING SUGARS BY LEAD OXALATE.—Oxalates were included by BRYAN (**2**) among the salts causing the precipitation of reducing sugars in the presence of basic lead acetate. In a previous paper (**5**) it has been shown that reducing sugars are co-precipitated in the presence of basic lead acetate with any amorphous precipitate, but that they are not precipitated to an appreciable extent in the absence of the lead-oxide radicle. This is illustrated in table II, where the reducing-sugar content of various sugar solutions and plant extracts treated with 1 and 5 cc. of 1.25 sp. gr. neutral lead acetate is compared after removing the excess of lead with potassium oxalate. Because of slower crystallization in the heavier precipitate, the volume of the lead oxalate from the 5 cc. treatment was about ten times that of the 1 cc. The last column of table II shows that there was no significant loss from either the excess of neutral lead acetate used or the heavy lead-oxalate precipitate.

TABLE II

LEAD OXALATE AS A CO-PRECIPITANT OF REDUCING SUGARS

SAMPLE	REDUCING SUGARS		RECOVERY WITH EXCESS TREATMENT
	1 CC. NEUTRAL LEAD ACETATE + POTASSIUM OXALATE IN EXCESS	5 CC. LEAD ACETATE + POTASSUM OXALATE IN EXCESS	
	mg.	mg.	Per cent.
Mangel extract no. 3	43.2	43.9	101.63
Mangel extract no. 4	72.9	72.4	99.31
Mangel extract no. 2	28.9	28.8	99.65
Mangel extract no. 6	41.3	41.6	100.76
Sweet potato extract	56.3	55.5	98.58
Celery leaf extract	154.2	154.2	100.00
Celery stalk extract	29.0	29.8	102.76
Tomato leaf extract no. 1	40.2	40.1	99.75
Tomato leaf extract no. 2	26.1	26.0	99.62
Applewood extract no. 3	93.2	91.7	98.39
Applewood extract no. 2	158.9	157.6	99.18
Pure fructose solution	183.0	182.4	99.67
Pure dextrose solution	132.1	130.8	99.01
Average	81.48	81.14	99.87

POTASSIUM OXALATE AS A LEAD PRECIPITANT.—If hydrogen sulphide is passed through a cleared solution deleaded with potassium oxalate, a black precipitate of lead sulphide is formed, indicating the incomplete removal of lead. Quantitative tests showed that of 184.8 mgs. of lead added to a mangel extract in 1 cc. of neutral lead acetate, 81.8 mgs. were carried down with the clearing precipitate and 100.8 mgs. were removed from the filtrate by the addition of an excess of potassium oxalate. Obtaining the lead remaining in the deleaded solution by difference, we find that 2.2 mgs. of lead or 2.14 per cent. were not removed. This incomplete removal of lead probably accounts for the loss in reduction when a solution deleaded with potassium oxalate is heated before making the reduction determinations. The data on the clearing of pure sugar solutions given in table I, show that the small quantity of lead left by potassium oxalate does not affect the reducing power of the solution under normal conditions. The same conclusion must be drawn from table III, where the reducing powers of solutions deleaded with potassium oxalate and hydrogen sulphide are compared.

TABLE III

A COMPARISON OF POTASSIUM OXALATE AND HYDROGEN SULPHIDE AS DELEADING REAGENTS

SOLUTION OR EXTRACT	LEAD ACETATE USED	DEXTROSE	
		DELEADED WITH POTASSIUM OXALATE	DELEADED WITH HYDROGEN SULPHIDE
		mg.	mg.
Applewood extract	2 cc. neutral	43.2	43.2
Tomato extract	1 cc. neutral	26.1	25.9
Mangel extract	1 cc. neutral	28.9	28.6
Mangel extract	5 cc. basic	33.9	33.5
Sweet potato extract	5 cc. basic	54.2	53.0
Dextrose solution	5 cc. neutral	130.8	130.6

Summary and recommendations

Potassium oxalate is a convenient deleading reagent for use in clearing plant extracts. It leaves the cleared solution neutral to litmus and an excess considerably greater than that required to give rapid flocculation of the lead-oxalate precipitate, has no effect on the reducing power of the solution.

Potassium oxalate does not precipitate all of the lead present and solutions deleaded with this reagent cannot be heated without lowering their

reducing power. Under normal conditions, however, solutions deleaded with potassium oxalate give very slightly higher reducing values than do those deleaded with hydrogen sulphide, which removes practically all of the lead, because the lead-oxalate precipitate is more easily washed than is the precipitate of lead sulphide.

It is recommended that a quantity of potassium-oxalate crystals, which is estimated to be 0.2 to 0.5 gms. in excess of the quantity necessary to remove the lead from the solution being cleared, be placed in a beaker and the cleared solution be filtered onto the crystals. This method insures rapid deleading and the high initial concentration of oxalate coupled with the time required to filter and wash the clearing precipitate allows for a partial crystallization of the lead oxalate so that it may be rapidly filtered and washed.

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AQUEOUS EXTRACTS OF SEEDS AS AGENTS IN THE PREPARATION OF SILVER SOLS

E. V. Miller and R. P. Hibbard

Introduction

Many methods have been suggested for the preparation of colloidal metals by condensation with the use of organic compounds as stabilizers (**1**). Among these compounds may be mentioned gum arabic, gelatin, sugar, glycerol, sodium citrate, saponin, barium arabinate, sodium protalbinate, and sodium lysalbinate.

Probably colloidal silver has received as much attention as has colloidal gold. Wool-fat has been employed as a stabilizing agent in preparing colloidal silver from organic solutions. Kohlschütter (**2**) reduced AgOH by means of hydrogen. Lüppo-Cramer (**4**) obtained a series of beautifully colored silver sols by the reduction of $AgNO_3$ with hydrochinone in the presence of gelatin. Carey Lea (**3**) reduced $AgNO_3$ with ferrous citrate, dissolving the deposit in water and reprecipitating with ammonium nitrate.

Svedberg (**6**) observed that a silver plate submerged in water or alcohol produced a silver colloid when illuminated by ultra-violet light or by X-rays. According to Traube-Mengarini (**7**) a certain amount of silver colloid may be produced by boiling silver in water.

Nordensen (**5**) showed that silver is oxidized by both water and alcohol and is dissolved as AgOH or some other compound. This silver solution, Svedberg has shown, may be reduced by illumination, while Traube-Mengarini produced similar results with traces of reducing agents. The dissolution (or oxidation) is accelerated by light, especially ultra-violet light.

The method for preparing colloidal silver described in this paper depends upon the ability of aqueous extracts of seeds to reduce $AgNO_3$ solutions. A number of methods for preparing colloidal silver have already been mentioned. This is an addition to the list and is highly recommended because of its simplicty of manipulation. It should therefore be of more than passing interest to plant physiologists, botanists, and chemists.

Methods

One gram of timothy seeds is stirred into 100 cubic centimeters of distilled water and allowed to stand about an hour. The solution is filtered and two drops N/10 $AgNO_3$ added. If the solution is then permitted to stand in diffused light for about half an hour a dark brown color will appear, this being due to colloidal silver. The speed of this reaction may

be increased by exposure to sunlight. However, too great exposure will precipitate the silver. If the aqueous extract is placed over a Bunsen burner and heated as soon as the $AgNO_3$ has been added, the colloidal silver will form within a few minutes.

If the colloidal silver solution is not placed in the strong sunlight the sol remains indefinitely stable. A solution in the laboratory at present has held up for over nine months.

Seeds investigated

The following seeds were later employed for reducing the silver, positive results being obtained in all cases except clover: Pea, bean, tomato, corn, wheat, buckwheat, grass, clover, sunflower, lettuce and beet. Later investigation revealed that though there was a precipitate formed in the clover solution, the supernatant liquid was colored a faint brown like that of other sols.

In a subsequent experiment colloidal silver solutions were prepared from the following: Corn, oats, wheat, rice, peas, beans, soy beans, cotton, beet, and gladiolus (bulb). In this case 50 and 100 seeds were soaked in 100 cubic centimeters of water. These were all placed under the ultramicroscope and were found to exhibit Brownian movement. In the sol prepared from the oats the particles were so numerous that the field presented the appearance of a confused mass of seething particles.

The particles of course are electrically charged, as was demonstrated by use of two platinum electrodes, placed on each side of the ultra-microscopic field and connected to storage batteries by means of a key. If the circuit were closed while the field was being observed the charge of the particles could be determined by their behavior in the electric field. All of the sols described possessed negative charges.

An outstanding feature of these colloidal solutions is the elaborate coloring. Colors varying from a dark amber to a rich rose or orchid were obtained. It is possible that different classes might be characterized by the color of their colloidal solutions. Corn, wheat, oats and rice all produced colloidal silver in a rose-colored suspension. Peas, beans, and soy beans were all characterized by a brown color. Other seeds were examined but could not be compared because no other closely related groups were included. These differences in color, especially the roseate hues, may depend in part on adsorption by the colloidal silver particles, as the usual color of silver sols is.

In an effort to locate the exact source of this reducing substance different parts of bean seeds were soaked in water and the solutions treated with $AgNO_3$. Beans were soaked in distilled water over night. The next morning they were taken from the water, the seed coats removed, and the plumule

and hypocotyl cut out of the cotyledons. These three parts (seed coat, cotyledons, and hypocotyl and plumule) were again soaked over night. On the following morning $AgNO_3$ was added to the extracts.

The intensity of the colloidal silver color was greatest with the seed coats, next with the cotyledons, and least with the hypocotyl and plumule. In the last the color did not form at all until after several hours.

Nature of the reducing substance

About ten cubic centimeters of the aqueous-extract of timothy seed were enclosed in a collodion sack and lowered into a beaker of distilled water. Two or three drops of N/10 $AgNO_3$ were added to this distilled water and in a few days the extract within the collodion sack had assumed a brown color characteristic of the colloidal silver. This would indicate non-diffusibility on the part of the reducing substance.

A more concentrated solution of the timothy extract presents a dark brown color, indicative of tannin. In the presence of ferric chloride a faint blue-black precipitate is formed, the intensity of the color resembling that produced by a .001 per cent. tannin when treated in the same way.

Following this a timothy extract was treated with lead acetate to remove the proteins. The excess lead was removed with H_2S. A few drops of $AgNO_3$ were added and a brown colloid, similar to the original, was formed. However, the colloid soon settled out.

The experiment was repeated, first adding enough tannin to make it a .001 per cent. solution. The same brown colloid was formed, appearing permanent at first, but settling out after a longer interval. The filtrate did not reduce Fehling's solution.

The instability of this last colloid was no doubt largely due to the presence of acid formed by the method employed for removing the excess lead. Later sodium carbonate was used instead of the hydrogen sulphide and the filtrate treated with $AgNO_3$. This time a colloidal silver formed which was not quite the same color as the original. This colloid lasted longer than the others (about a week) but finally settled out like the rest.

Difficulty in identifying this reducing substance arises from the fact that many substances are extracted from the seeds by both water and alcohol. In some instances sugar has been found present and in others it has not.

An alcoholic (90 per cent.) extract of corn meal has been found to reduce the silver nitrate, producing a colloid identical in appearance with the original one. In previous cases the reduction has been brought about by an aqueous extract. Furthermore, this alcoholic extract has produced a silver sol which thus far rivals the aqueous extract in stability.

In another experiment an alcoholic extract of corn meal was evaporated and the alcohol replaced with water as the alcohol evaporated. The zein was precipitated in this process. This impure zein was dissolved in ethyl alcohol and $AgNO_3$ added to the solution. A silver sol, very similar to the original, was formed. After a period of a week this sol became cloudy and appeared to be precipitating.

From the above results it seems possible that both zein and sugar would be extracted by the alcohol. When the alcohol is replaced by water the sugar would go into solution, leaving the zein to be precipitated. Hence it might be surmised that the silver can be reduced by either the alcohol- or water-soluble proteins and the sol stabilized by sugar or *vice versa*.

An aqueous extract of corn meal was made after the meal had first been extracted with 90 per cent. alcohol. This aqueous extract reduced Fehling's solution when tested for sugars. There were also proteins present which were removed before testing for sugars. A colloidal silver solution was produced by this aqueous extract but the sol was unstable.

The zein mentioned in this paper has been designated as "impure zein." It was prepared by extracting corn meal with alcohol, evaporating off the alcohol, replacing the alcohol with water as the alcohol evaporated, and collecting the zein as it precipitated.

A little later some pure zein was prepared. An alcoholic extract of corn meal was made, the zein salted out with dilute NaCl, and the NaCl removed by dialysis. It is possible that some of the carotinoid pigments were carried down with the zein but we shall distinguish between the two by calling this last one "pure zein."

A stable colloid has been prepared by dissolving this "pure zein" in alcohol and adding the $AgNO_3$. With the impure zein the colloid was not stable unless a small amount of arabinose was added.

Summary

A simple method has been described for preparing colloidal silver from aqueous extracts of seeds. The process is essentially one of reduction or condensation.

Further investigation revealed three other methods for preparing colloidal silver. They are as follows: (1) Alcoholic extract of seeds; (2) Alcoholic solution of pure zein; (3) Alcoholic solution of impure zein plus arabinose.

The reducing substances are not as yet known but it seems probable that they might be alcohol- and water-soluble proteins with sugar as the stabilizer.

The work on this problem has been made possible by the encouragement and financial aid of the D. M. Ferry Seed Company of Detroit.

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BRIEF PAPERS

CLIMATIC EFFECTS IN THE METABOLISM OF MAIZE*

These brief notes relate to preliminary results with maize by methods similar to those used in a previous study of the sugar beet.[1] Particular interest attaches to the investigation of this plant because of its comparative independence from climatic effects in composition of the grain, as previously shown.[2]

In the present test composite samples of leaf, stalk and ear tissue were taken from fifty plants six times in a period of ten days during mid-August. These were preserved in hot alcohol, with the addition of calcium carbonate. The extract was recovered in the usual manner and both extract and residue were subjected to chemical analysis by conventional methods.[3] In view of its limited scale, detailed presentation and discussion of the work would be untimely, but certain results seem to be of considerable significance.

The percentage of sucrose in the dry matter of the leaf greatly exceeded that of reducing sugars. Both sucrose and dextrins varied in general with the solar radiation. Sugars in the stalk and ear varied independently of their proportions in the leaf, and the same was true of mono-amino acids. On the other hand, the composition of the stalk was relatively stable and correlated with that of the ear.

The accompanying table gives a comparison of composition in leaves of maize with that of other species grown adjacent in the field with similar cultural treatment and sampled simultaneously. Varieties used were: Yellow Dent maize, Marquis wheat and Black Amber sorghum.

Despite its relative maturity (early heading stage) the wheat contained much higher proportions of total soluble nitrogen and of protein nitrogen than did maize. On the other hand, sorghum had much the same composition as maize. This is in harmony with its relative stability of composition with varying climate.

Of the two types of composition in tissue adapted largely to synthetic processes one would expect that which included the more stable compounds

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[1] TOTTINGHAM, W. E., LEPKOVSKY, S., SCHULZ, E. R., and LINK, K. P. Climatic effects in the metabolism of the sugar beet. Jour. Agric. Res. 33: 59–76. 1926.

[2] TOTTINGHAM, W. E. Physiological stability in maize. Science (New Series) 59: 69–70. 1924.

[3] Official and tentative methods of analysis, Association of Official Agricultural Chemists. Washington, D. C., 1919. pp. 5–7, 78–79, 81, 95–96.

TABLE I

COMPARATIVE COMPOSITION OF LEAVES

PLANT SPECIES	DATE OF SAMPLING	DRY MATTER	TOTAL NITROGEN IN DRY MATTER	PARTIAL DISTRIBUTION IN THE TOTAL NITROGEN	
				TOTAL SOLUBLE N	SOLUBLE PROTEIN
		Per cent.	Per cent.	Per cent.	Per cent.
Maize	7/15/24	25.7	3.8	18	5
Wheat	7/15/24	41.5	3.4	35	15
Maize	7/17/24	23.1	3.7	27	14
Wheat	7/17/24	34.8	3.1	55	32
Maize	7/16/25	27.2	3.5	26	14
Sorghum	7/16/25	24.4	4.0	30	17
Maize	7/22/25	27.2	3.5	37	26
Sorghum	7/22/25	26.0	4.1	41	27

to be the more resistant to disturbance by climatic variations. Maize and sorghum leaves possess the common factor of low proportions of total soluble nitrogen and soluble protein, as contrasted with high values of these factors in wheat and the sugar beet. Furthermore, maize is characterized by low proportions of reducing sugars, while the sugar beet leaf contained much more reducing sugar than sucrose in our tests. MILLER'S[4] results show the same status of sugars in sorghum as in maize, while such data as we have from greenhouse cultures place wheat in the category of the sugar beet.

There are not sufficient grounds for imputing to this difference in composition the chief cause of stability of corn and sorghum against climatic influences. On the other hand, it may be ultimately important to consider that sucrose and less soluble forms of protein are quite likely to be less altered by the effects of temperature and other climatic factors than are such reactive compounds as glucose, soluble proteins and other soluble nitrogenous compounds.—W. E. TOTTINGHAM and H. W. KERR, *The University of Wisconsin.*

[4] MILLER, E. C. Daily variation of the carbohydrates in the leaves of corn and the sorghums. Jour. Agric. Res. 27: 785–808. 1924.

A DEVICE FOR MAINTAINING CONSTANT LEVEL OF CULTURE SOLUTIONS

(WITH ONE FIGURE)

In growing certain woody plants in continuously flowing culture solutions it has been found essential to submerge only a portion of the established root system while still maintaining a relatively high humidity around the unsubmerged roots. The following apparatus has been found especially satisfactory for this purpose, allowing the use of ordinary jars of glass or other material as culture vessels without alteration. The arrangement in use is shown in the accompanying figure.

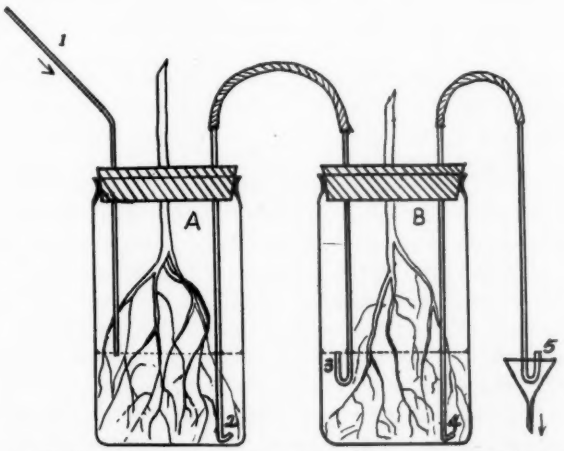

FIG. 1. Constant level device for culture solutions. Description in text.

The tube 1 leads from a supply reservoir into jar A, ending near the surface of the solution. The tube 2–3 is a siphon having a j-bend at 3 with the shorter arm 2 or 3 inches long. Tube 4–5 is a similar siphon leading from jar B and emptying into a receiving jar. The ends of the siphons at 2 and 4 may be at any level below the openings at 3 and 5, but are shown extending to the bottoms of the jars, an arrangement which aids in the circulation within the jars, since the inflow occurs at the surfaces of the solutions. The short bends in the tubes at 2 and 4 are to prevent entrance of gas bubbles which would break the siphon streams.

When the jars are filled to the levels shown and the siphons are filled and solution is allowed to enter A at the desired rate through tube 1, it automatically flows through the siphon into B, whence it is likewise removed through the second siphon and passes into the receiver. If the solution stops entering from tube 1, the whole system stops but the siphons do not break on account of the j-bends at 3 and 5; flow starts again automatically when solution flows into A. The level of solution in A and B is determined by the height of the openings at 3 and 5. The level of solution in B may be maintained the same or lower than that in A, but not higher. As many jars as desired may be operated in series.—F. G. ANDERSSEN, *Pomology Laboratory, University of California.*

NOTES

International Congress of Plant Sciences.—The Ithaca Congress has passed into history, and is generally conceded to have been the most valuable meeting of botanists ever held in America. The meetings had been planned with the utmost care, and every feature of the great gathering evidenced the thoughtfulness and thoroughness with which the preparations had been made. The attendance far exceeded expectations and fell but little short of 1,000. The attendance from foreign lands made only a fraction of the total, but was very satisfactory. Everyone regretted the absence of the French botanists from the meeting but nearly all the important nations were represented by delegates. It was particularly gratifying to see the large Russian delegation at the Congress.

Although no legislation was attempted at this Congress, it has paved the way for other international gatherings, the next of which will be held in London in 1930.

The physiological meetings of the Ithaca Congress were very well attended, and the papers presented were generally of high merit. It is a pleasure to be able to present a fairly full account of the physiological proceedings to our readers in this number of PLANT PHYSIOLOGY.

Society Dues.—The constitution of the American Society of Plant Physiologists provides for a fiscal and official year beginning July 1, and ending June 30. The journal year, however, begins January 1. The dues paid during the last half of 1925 entitle the member to all of the issues of PLANT PHYSIOLOGY for 1926, and the dues now payable are for the 1927 issues of the journal, and for membership from July 1, 1926, to June 30, 1927. By this arrangement the secretary-treasurer has six months in which to prepare the mailing list for the following calendar year. New members joining the Society at this time should remit $10.00 if they desire to secure the first volume of PLANT PHYSIOLOGY. All physiologists are urged to secure the first volume now, as it will soon be unobtainable in complete sets.

Protoplasma.—An international journal of the physical chemistry of protoplasm is announced by Gebrüder Borntraeger, of Berlin. The ground to be covered includes the colloid chemistry of protoplasm; physico-chemical properties of protoplasts, such as surface tension, viscosity, plasticity, swelling, elasticity, adhesion, adsorption and acidity; microchemistry and electrometry of protoplasts; structure of living protoplasm; osmotic phenomena of protoplasts; permeability, plasmolysis, narcosis, cytolysis, hemolysis, vital staining; physico-chemical causes of protoplasmic movement; microdissec-

tion; ultramicroscopic and polarization studies of protoplasm; mechanism of cell and nuclear division; protoplasmic activation; physico-chemical problems of pharmacological and toxic effects; resistance and sensitiveness to radiations; physical-chemistry of pathological cells, as of tumors; and studies of non-living colloidal models which throw light on protoplasmic behavior.

The editors are JOSEF SPEK, of the Zoological Institute, Heidelberg University, and FRIEDL WEBER, of the Plant Physiological Institute, University of Gratz. At present manuscripts should be sent to Dr. F. WEBER only.

We extend good wishes to the new journal. Plant physiologists will no doubt find much valuable material appearing in Protoplasma, and need it in both private and public libraries.

Author Abstracts.—The editor of Biological Abstracts has requested the cooperation of PLANT PHYSIOLOGY in securing abstracts for the papers appearing in this journal. The simplest and quickest way to have the abstracts appear is to have author abstracts accompany manuscripts when they are first submitted for consideration by the editors of PLANT PHYSIOLOGY. In order to assist the editors of Biological Abstracts in the enormous task of securing abstracts of all botanical literature, we are therefore requesting all authors of papers to furnish with the manuscript submitted a brief, accurate abstract which may be forwarded to Biological Abstracts at the time of publication. This will insure the earliest possible appearance of the abstracts, and should provide better, more intelligent abstracts than any other method. The main difficulty is that authors sometimes think their material is much more valuable than it is, and make abstracts too long and detailed. The abstracts should be very brief, and right to the point. Most papers can be adequately abstracted in 100 to 200 words. We expect all of our contributors to comply with this request.

The University of Minnesota Section.—Permanent organization of the University of Minnesota Section of the American Society of Plant Physiologists is reported as follows: Local vice president, Professor A. C. ARNY, University Farm, St. Paul, Minnesota; second vice president, Professor L. O. REGEIMBAL; secretary-treasurer, Professor A. H. LARSON. In accordance with the constitutional provision, Professor ARNY becomes *ex-officio* a member of the Executive Committee of the Society. The new section has the cordial good wishes of all who are interested in strengthening the bonds of good fellowship, and encouraging the resourcefulness of plant physiologists in carrying forward their research.

Photosynthesis.—It is a pleasure to record the publication of a book by this title from one of the best known students of the chemistry of the process, Dr. H. A. SPOEHR, of the Carnegie Institution. The book takes up particularly those phases of the subject which look toward the explanation of the plant's unique power to act as a converter of radiant sunlight energy into potential chemical energy of food and fuel. Inasmuch as it is difficult to draw many conclusions in the present state of our knowledge, the author gives more attention to the results of experimental investigations of the chemistry of the process than to the conclusions which have been derived from field observations and empirical studies. The book is suggestive, as the author gives special attention to features which offer promise of results along experimental lines. All physiologists will want this excellent book. It is published by the Chemical Catalog Co., New York. Price, $6.50.

Colloid and Capillary Chemistry.—A particularly valuable service has just been rendered by H. S. HATFIELD in translating the third German edition of FREUNDLICH'S Colloid and Capillary Chemistry into the English language. This will make more available one of the truly great works in this field. It is an excellent translation of FREUNDLICH'S masterpiece, and needs no description. The book, 883 pp., price $14.00, is published by E. P. Dutton and Co., New York.

Surface Equilibria.—Attention is directed to the publication of a book on Surface Equilibria of Colloids by P. LECOMTE DU NOÜY. This book will be of considerable interest to physiologists generally, from the interesting and valuable technique which DU NOÜY has developed, and from the rather far reaching conclusions which he draws with reference to the colloidal systems of organisms as the result of his measurements of surface equilibria. The Chemical Catalog Co., New York, is the publisher. Price, $4.50.

Physikalische Chemie der Zelle und der Gewebe.—The sixth edition of this indispensable work has appeared from the Engelmann Press, Leipzig, 1926. Nearly all the chapters have been modified somewhat by the rapid advances made since the fifth edition was published two years ago.

Physical Chemistry and Colloid Chemistry.—A condensed introduction to the physical and colloid chemistry most needed by students of plant and animal physiology and medicine, with the mathematics made unusually clear, has been prepared in German by the recent translation of KRUYT'S little book from the second Dutch edition. It is translated by NOWAK, and published by the Akademische Verlagsgesellschaft, Leipzig, 1926. This little Einführung in die physikalische Chemie und Kolloidchemie will prove

very helpful in getting hold of the fundamentals. Incidentally it furnishes good practice in reading German. It is clearly written, and not too difficult.

Plant Nutrition and Plant Production.—Under this title the University of California Press has published the Hitchcock Lectures of 1924, which were delivered by Sir JOHN RUSSELL, Director of the Rothamsted Station in England. There are five chapters to this excellent book, as follows: The study of plant nutrients; positive science and exact demonstration; decay and the living plant, *mors janua vitae*; the soil microorganisms, can they be controlled and utilized; and the soil and the living plant. The historical introduction follows the lines of Soil Conditions and Plant Growth. As is true of all of Sir JOHN RUSSELL'S writings, the story of plant nutrition is fascinatingly told. Plant physiologists will all want to read this delightful summary, written by one of the world's eminent scientists. The book is beautifully illustrated, with 37 figures and 21 full-page plates. The plates alone are worth the price of the book. There is a vast deal of sound philosophy incorporated into the story, for Sir JOHN RUSSELL is more than an investigator; he is a student of life in its broadest aspects, and a philosopher of unusual keenness of insight. This book should have a wide circulation. The price is $2.50, and can be obtained from the University of California Press, Berkeley, California.

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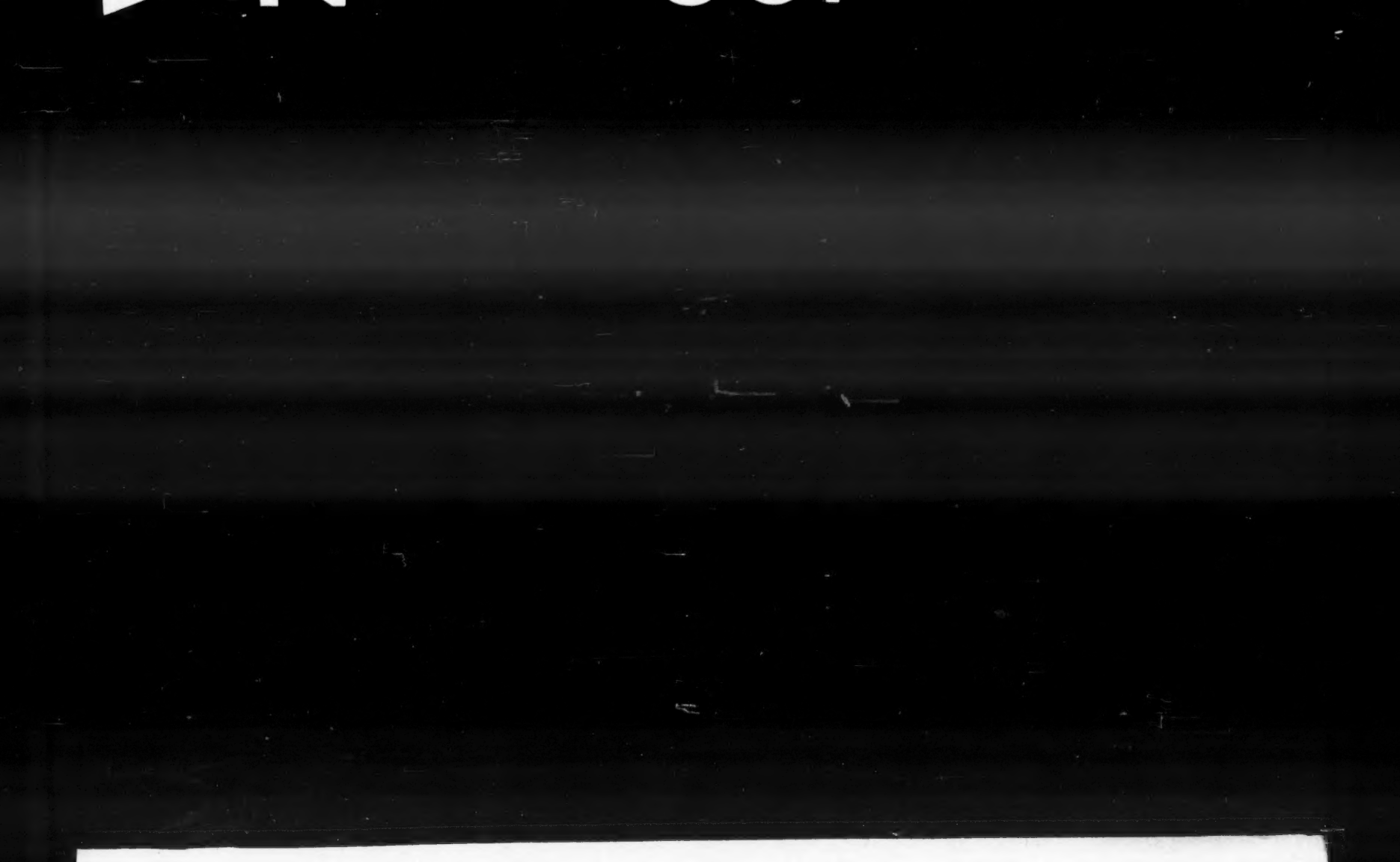
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